THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXVIII NOVEMBER-DECEMBER, 1952 NUMBER 6

THE IDENTIFICATION OF TYPES OF PULMONARY CANCER IN CYTOLOGIC SMEARS *

NATHAN CHANDLER FOOT, M.D.

(From the Laboratory of Exfoliative Cytology, Department of Anatomy, Cornell University Medical College, New York 21, N.Y.)

During the developmental period of cytologic technics for diagnosing pulmonary cancer in smears of sputum and bronchial washings, roughly the 7 years from 1945 to 1952, it has become increasingly evident[1] that it is possible to determine not only the presence of cells exfoliating from malignant pulmonic tumors, but also to make approximately accurate estimates as to the type of new growth involved. The practical application of this observation is manifest: not only can the presence of cancer be detected definitely in a very large percentage of the cases in which it is harbored, but its type may be recognized in 80 per cent of these. This affords a basis for prognosis as well as diagnosis. Should a pulmonary carcinoma be detected and be believed to be secondary to a primary growth still situated elsewhere in the body, operation on the lung would be futile; should it appear to be a primary type of relatively low-grade malignancy, on the other hand, operation would afford a chance of possible cure.

With these points in view a series of smears from 341 cases of pulmonary cancer was chosen for study. These included only those cases in which the laboratory diagnosis on smears was "class IV" or "class V" (fairly conclusive and conclusive evidence of malignant neoplasm, respectively) and in which sections of surgical or post-mortem material were available. They were selected from a series of smears of sputum and bronchial aspirates submitted to the laboratory during the past 7 years by several hospitals and physicians. Although a somewhat greater number of cases was available, only the first 5,000 were used as a source of material; these were generously supplied by the laboratory for use in this study.

The majority of the cases finally selected came from New York

* Aided by a grant from the Cancer Control Division of the U.S. Public Health Service. Received for publication, May 3, 1952.

Hospital and Memorial Hospital. The series of smears thus set up was carefully studied and correlated with sections from surgical specimens or from specimens taken for biopsy from the actual tumors; any case in which the diagnosis from smears could not be checked against that on sections of tissue was forthwith discarded. The original diagnoses on the smears chosen for examination and study had been made by members of the staff of the laboratory and had been almost invariably corroborated by Dr. Papanicolaou.

The purpose of the investigation was to ascertain with reasonable accuracy the percentage of cases in which the type as well as the presence of pulmonary cancer could be determined. Diagnoses were based on the morphologic features of exfoliated cells in smears, all of them being checked against those of cells in sections of the tumors after they have been removed, or sampled by biopsy. It will be shown that the degree of accuracy for typing malignant tumors is satisfactorily high, ranging from 69.2 per cent for the most difficult to 89.9 per cent for the most readily recognized type.

Types of Malignant Pulmonary Tumors

Before detailing the results of the investigation, it will be necessary to define the various types of malignant pulmonary growths and the nomenclature to be employed in this paper. The more one studies bronchogenic carcinoma, the more one is convinced that the classes into which it may be subdivided are not entirely clear-cut; they tend to overlap at their extremes, but there is enough adherence to type to warrant attempting recognition. For example, some tumors will exhibit squamous differentiation and keratinization of cells in isolated foci without being typically epidermoid; this may be noted in pleomorphic and adenomatous types. Anaplastic carcinoma may consist chiefly of small round cells, which may be oat-shaped in sections, but they may occasionally exfoliate larger cells that arouse suspicion of pleomorphic or epidermoid carcinoma. In one instance such a tumor, definitely identified as anaplastic in sections, showed so many abnormal, keratinized, large and distorted cells in smears, that it was diagnosed both by the laboratory staff and by me as epidermoid and the infrequent clusters of typical anaplastic cells were overlooked. Fortunately, overlapping of types is relatively rare. McDonald and his collaborators[2] have made it quite plain in their recent paper that there is significance to the various cellular types of bronchogenic carcinoma; that certain forms are more usually situated centrally in the larger bronchi, while others tend to appear oftener in the periphery of the lung. They also noted the variation of sex incidence in the patients in

their series, which is largely influenced by the type of tumor involved; about 3 per cent of patients with epidermoid or anaplastic carcinoma are women, whereas from 14 to 20 per cent of those with pleomorphic carcinoma or adenocarcinoma are of that sex. The figures in the present investigation confirm these to a large degree.

Bronchogenic Carcinoma

The largest and most important group of primary tumors of the lung is comprised of growths taking their origin from the bronchial mucosa or the parabronchial mucous glands. It is roughly subdivisible into six types.

Epidermoid (Squamous Cell) Carcinoma. The epidermoid carcinoma resembles epidermoid structures like skin or stratified squamous mucosa and exhibits a variable degree of keratinization. Most important is its general resemblance to skin, whence its name.

Pleomorphic (Large Cell) Carcinoma. There is no resemblance to normal tissue in the pleomorphic type which comprises large cells which may be uniform or very variable in size, shape, and appearance, the last feature being frequently bizarre in the extreme. The cells are usually loosely associated in masses that tend to undergo central necrosis which may lend to the tumor a gland-like appearance. They show no keratinization, which distinguishes them from the epidermoid group. It is probable that they constitute a very immature, distorted, and malignant variety of the epidermoid type, but this cannot be stated categorically.

Adenocarcinoma. Adenocarcinoma of the lung apparently arises from parabronchial mucous glands. Ewing[3] and McDonald *et al.*[2] believed this to be so, as I do. Some authorities, however, think that they may develop from goblet cells of the bronchial mucosal lining. It is often difficult to distinguish primary bronchogenic adenocarcinoma from secondary metastatic adenocarcinoma coming to the lungs from some other organ. The primary form resembles mucous adenocarcinoma in general.

Anaplastic (Small Cell, Oat Cell) Carcinoma. The anaplastic carcinoma is composed of small round or ellipsoidal cells which appear to be extremely immature or embryonal and are uniform in their morphologic characteristics. In sections they may be elongated and oat-shaped, hence the popular name given them by Adami.

Carcinoma in Situ. A relatively recent arrival to the ranks of tumor terminology is the non-invasive carcinoma, or carcinoma ***in situ,*** a growth in which there are local areas of distinct metaplasia and vigorous mitotic activity in the bronchial mucosa without, however, visible

invasion of the supporting tissue. In this series there were several examples of apparently advanced stages of this type, in which invasion was already proceeding, so that the terms non-invasive or *in situ* became inappropriate. Nevertheless, the morphologic features of bronchial mucosa were so well retained that the tumors could be called neither epidermoid nor pleomorphic. For the purpose of convenience they were noted as "bronchial" types of epidermoid carcinoma, which was considered justifiable on the basis that epidermoid tumors often present areas in which bronchial architecture persists more or less. Furthermore, if this were not done, another and possibly impure type would have to be added to the already long list of varieties.

Bronchial or Tracheal Adenoma (Carcinoid, Mixed Tumor). Bronchial adenoma was, for a long time, considered to be non-malignant, but it is coming to be regarded as a slowly growing, but ultimately malignant neoplasm. It consists of small round cells that are granular and may be arranged either in solid masses resembling those of the intestinal carcinoids, even to possessing argyrophilic granules, or may take on the pattern and appearance of the cells of those primitive salivary gland carcinomas sometimes called oncocytomas. Its recognition in smears is difficult to the point of being well-nigh impossible, as it does not exfoliate extensively and its cells are readily confused with those of anaplastic carcinoma. Only two of these tumors occurred in this series and both were unrecognized in smears.

ALVEOLAR CELL (TERMINAL BRONCHIOLAR) CARCINOMA

Alveolar cell carcinoma is comparatively rarely encountered. It apparently arises in the alveolar or terminal bronchiolar epithelium and, when found in smears of exfoliated material, is only fairly characteristic in its appearance. Sections of tissue show areas in which the alveoli are lined with grotesque palisades or festoons of metaplastic epithelium which transform it into the semblance of an adenocarcinoma. Since other carcinomas, whether bronchogenic or metastatic, may invade pulmonary alveoli and their cells align themselves along the walls, there is always an element of doubt in making a positive diagnosis of this particular tumor.

METASTATIC CARCINOMA

Certain tumors of other organs are prone to metastasize to the lung, *i.e.*, pancreatic, mammary, gastric, and intestinal carcinomas. They are usually fairly readily recognized as aliens in smears, as they retain much of their original structure which is quite unlike that of primary pulmonic tumors. Most of them are adenocarcinomas, either definitely glandular in appearance or of solid "simplex" type.

SARCOMA

Primary pulmonary sarcoma, arising from fibrous or cartilaginous tissue, is uncommon; there was but one example in this series. The appearance of the growth is naturally similar to either distorted fibrous or cartilaginous tissue and, as these are widely distributed throughout the body, there is always a question as to whether a given pulmonary sarcoma is primary or secondary.

TERATOMA

Intrathoracic teratoma may erode into a bronchus and discharge cells which will appear in the secretion; like other teratomas they are characterized by the mixed nature of their architecture and components, and their resemblance to poorly developed embryos. In their malignant form they are composed of undifferentiated tissue that looks like mesoderm, in which may be embedded islands of more or less mature epidermis, glands, bone, cartilage, or nervous tissue. If a teratoma shows any areas of undifferentiated, rapidly dividing cells, it should be considered malignant.

Results of the Investigation

The total number of cases investigated, after rejecting unconfirmable material, was 341; of these, 8 were epidermoid carcinomas of the esophagus, which should be omitted from the statistical reckoning on that account. This tumor exfoliates cells that reach the mouth and may be expectorated, or it may break through into the trachea or a bronchus, whereupon masses of its cells mingle with the secretion which is coughed up. In diagnosing such tumors as epidermoid carcinoma, no mistake was made. Some of them were actually recognized as esophageal because of their hyperkeratinization and the occasional epithelial pearls, not to mention clues from clinical histories.

The results of the study were tabulated on work sheets with the number of the smear, that of the surgical specimen, the patient's name, the source of the smear (whether sputum or bronchial aspirate), the laboratory diagnosis of the smear, that of the surgical specimen, and my diagnoses of smear and specimen. Diagnoses were listed as general, specific, or erroneous as the case might be. It will be noted that the laboratory diagnoses, which were made by various members of the staff and checked by Dr. Papanicolaou, were more often general than specific, being usually limited to "fairly conclusive" (class IV) or "conclusive evidence of malignant neoplasm" (class V). My diagnoses were, in contrast, usually specific, as I was attempting to be so even at the cost of a good score for accuracy. The laboratory can take no such

chances; specific diagnoses as to types are more or less discouraged and the policy is to issue a general diagnosis that is supported by the evidence and which will warn the physician in charge of the case of what he may expect and how to proceed.

It may also be pointed out that the recognition of types of pulmonary cancer is more likely to be an easier matter for an experienced surgical pathologist than it would be for a younger and less experienced man or for a cytologist who has not studied these tumors in the gross or in microscopic sections rather than smears. The question in this particular investigation, however, is not "can anybody do it?" but rather "can it be done at all?"

After discarding the 8 cases of esophageal carcinoma, there still remained one in the New York Hospital series in which I regarded the diagnosis as tuberculous tracheitis and not carcinoma. This belief was based upon the discovery of numerous tubercles in the tracheal mucosa taken for biopsy and upon the absence of definitely malignant neoplastic cells in smears, of which there was a collection of about 40. This reduces the number of cases germane to the study to 331, the figure used for reckoning percentages of occurrence of the various types of tumor noted in its course.

EPIDERMOID CARCINOMA

Criteria for Recognition in Smears

The smears from cases of epidermoid carcinoma tend to be relatively clean and free from numerous leukocytes. The most important criterion is the presence in the smear of large, obviously squamous and keratinized cells, either as single examples or arranged in clusters. Papanicolaou's stain 267 (combining hematoxylin, eosin, orange-G, and light green) is best suited for accentuating the keratin content and it assists materially in making the diagnosis. The size of the cells, as well as their shape, may vary considerably; they may be attenuated into serpentine, sinuous forms, they may be squash-shaped or spheroidal. Some of them are evenly elliptical or cuboidal and small, if they come from erosions into the deeper layers of the neoplastic epithelium. Concentrically arranged clusters may constitute pearls, but as has been said, these are more frequently noted in esophageal than in pulmonary epidermoid carcinomas. Tight clusters of cuboidal cells cause confusion as they may suggest bits of the solid type of adenocarcinoma, or the anaplastic carcinoma, and they must be distinguished from these. The nuclei of all of these abnormal cells are intensely stained or pyknotic, may be large and pleomorphic, and they appear definitely metaplastic when compared with those of desquamated

bronchial or oral epithelium. Mitotic figures are noted infrequently and are of little value in drawing conclusions. There are also cells intermediate in size between the extremes just described. They have an oval or elliptical outline, a rather small, precisely stained nucleus, and a distinct affinity for orange-G, wherefore Papanicolaou calls them orangeophil cells. They are often helpful in diagnosing epidermoid carcinoma. Non-nucleated, degenerate "ghost cells" are abundant in the presence of pulmonary carcinoma, but are of little value in diagnosing types. Recognition of the type of tumor is easiest in those cases in which clusters abound in the smear. Keratinization may vary greatly; sometimes it is difficult to determine its presence. In these instances differentiation between epidermoid and pleomorphic carcinoma becomes difficult, which explains some of the errors made in diagnosing pleomorphic carcinoma in this series. More epidermoid carcinomas were misdiagnosed as pleomorphic than vice versa.

Findings

There were 175 bronchogenic carcinomas in the series. Only 6, or 3.5 per cent occurred in women, a ratio of about one woman to 30 men.

TABLE I

Data on Epidermoid Bronchogenic Carcinoma

Incidence in women		Incidence in total series		Diagnostic accuracy as to type		Laboratory diagnosis		Writer's diagnosis	
	per cent		*per cent*		*per cent*				
N. Y. H.*	3.90	N. Y. H.*	52.0	N. Y. H.*	91.0	S*	27	S*	71
M. H.	2.85	M. H.	52.0	M. H.	87.7	G	49	G	0
All	3.4	All	52.0	All	89.9	W	1	W	6

* N. Y. H., New York Hospital; M. H., Memorial Hospital. S, specific; G, general; W, erroneous diagnoses, respectively.

PLEOMORPHIC BRONCHOGENIC CARCINOMA

Criteria for Recognition in Smears

The smears of pleomorphic bronchogenic carcinoma tend to be "dirty," with many pus cells and much débris present, which may be explained by the tendency of this type to undergo necrosis. Smears show cells of widely varying size and shape, with distorted and overstained nuclei, but there is little of a positive nature to rely upon in making the diagnosis. It is rather by the absence of keratinization or of glandular clusters and particularly of uniformity in size, shape, and appearance that one formulates a conclusion. When clusters of large, irregular cells with overstained nuclei are noted, and the architecture of the clusters is that of a loosely connected group of cells without

definite arrangement the tumor is, in all probability, of the pleomorphic type.

Findings

There were 65 instances of pleomorphic carcinoma in the series; of these, 45 were correctly diagnosed, a rather poor showing. There were 11 female patients in the group, or 17 per cent, a ratio of one woman to 6 men.

TABLE II

Data on Pleomorphic Bronchogenic Carcinoma

Incidence in women		Incidence in total series		Diagnostic accuracy as to type		Laboratory diagnosis		Writer's diagnosis	
	per cent		*per cent*		*per cent*				
N. Y. H.*	17.4	N. Y. H.*	16.3	N. Y. H.*	78.0	S*	1	S*	17
M. H.	11.9	M. H.	21.0	M. H.	64.7	G	20	G	1
All	13.9	All	19.0	All	69.2	W	2	W	5

* N. Y. H., New York Hospital; M. H., Memorial Hospital. S, specific; G, general; W, erroneous diagnoses, respectively.

ADENOCARCINOMA

Criteria for Recognition in Smears

The criteria for diagnosing primary adenocarcinoma are also somewhat difficult to postulate: if the cell clusters exhibit a glandular arrangement, or there are several cells in a row (as though detached from a glandular complex), or if the cells are vacuolated, or if their nuclei are situated at one end, rather than in the center of the cells, one may suspect adenocarcinoma. The nuclei are hyperchromatic although rather more precise and delicate than those of the other types, and they show prominent nucleoli. If there are very abundant clusters of cells tightly packed in circumscribed masses, or in obviously glandular arrangement, the chances are that the tumor is a secondary metastatic growth rather than a primary pulmonic adenocarcinoma. The mammary duct-cell and simplex types retain their original architecture so well that they may usually be recognized as of mammary origin. Rectal carcinoma also may retain its mucous appearance, but pancreatic carcinoma produces solid, cord-like groups of cells without glandular lumina; once studied they become familiar and their diagnosis fairly certain.

Findings

There were 43 adenocarcinomas in the series, of which 6 were actually, or presumably, metastatic and therefore secondary growths. In 3 instances they were of pancreatic, in one probably of gastric, in

one mammary, and in one of uncertain origin. There were 16 women in this group, one with anamnestic and other indications of metastatic rather than primary adenocarcinoma; thus 15 women presumably had primary lesions, which is 35 per cent of the group, showing a ratio of one woman to 2 men.

TABLE III

Data on Adenocarcinoma of the Lung

Incidence in women		Incidence in total series		Diagnostic accuracy as to type		Laboratory diagnosis		Writer's diagnosis	
	per cent		*per cent*		*per cent*				
N. Y. H.*	14.3(0.0)	N. Y. H.*	14.9(12.0)	N. Y. H.*	90.5(88.9)	S*	3(3)	S*	32(31)
M. H.	27.2(20.0)	M. H.	11.0(10.0)	M. H.	72.7(70.0)	G	35(29)	G	1(0)
All	21.0(10.7)	All	12.5(10.8)	All	81.4(78.4)	W	5(5)	W	8(8)

* N. Y. H., New York Hospital; M. H., Memorial Hospital. S, specific; G, general; W, erroneous diagnoses, respectively. (The figures in parentheses are those obtained after eliminating metastatic growths.)

ANAPLASTIC (SMALL CELL, OAT CELL) BRONCHOGENIC CARCINOMA

Criteria for Recognition in Smears

In anaplastic carcinoma there is a copious exfoliation of small round cells with equally minute spherical nuclei and a narrow zone of surrounding cytoplasm which may be so pale as to be vacuolar in its appearance. The smear, as a whole, is untidy and shows much débris, often of a basophilic type. Clusters of anaplastic cells are often triangular and resemble miniature bunches of grapes, or they may be cylindrical or elongated ovoid in outline. The extended, heavily stained nuclei and practically negligible cytoplasm that characterize the cells when observed in sections of tissue are seldom seen and the blurred, pyknotic appearance of the nuclei also disappears. Indeed, one may study the cytology of this tumor much more intelligently and advantageously in smears than in sections. In smears of bronchial aspirates there are very often cylindroids, like renal casts; this is a mechanical effect of smearing the coagulated sediment onto slides, but when anaplastic carcinoma is present, such structures are found to contain more or less compact masses of the miniature cells, each about half the size of a polymorphonuclear leukocyte. Their dimensions may vary in a group, within narrow limits, which is quite characteristic and differentiates them from lymphocytes. The best indication of the presence of anaplastic carcinoma is the abundance of miniature cells. Mistakes in diagnosis are less frequently made with this type than with most of the other forms of bronchogenic carcinoma, but one must be extremely

careful in making the diagnosis from smears of bronchial aspirates, in which distorted, but actually normal, ciliated bronchial epithelium can often form clusters like those of this carcinoma.

Findings

There were 41 cases of anaplastic carcinoma in this series. Of these only 3 were in female patients, an incidence of 7.3 per cent.

TABLE IV

Data on Anaplastic Bronchogenic Carcinoma

Incidence in women		Incidence in total series		Diagnostic accuracy as to type		Laboratory diagnosis		Writer's diagnosis	
	per cent		*per cent*		*per cent*				
N. Y. H.*	6.6	N. Y. H.*	10.6	N. Y. H.*	93.4	S*	25	S*	34
M. H.	7.7	M. H.	13.0	M. H.	77.0	G	13	G	0
All	7.3	All	12.0	All	83.5	W	3	W	7

* N. Y. H., New York Hospital; M. H., Memorial Hospital. S, specific; G, general; W, erroneous diagnoses, respectively.

OTHER TYPES OF PULMONARY TUMOR

Criteria for Recognition in Smears

Carcinoma in Situ. As the accurate diagnosis of carcinoma *in situ* depends upon the presence of metaplastic changes in the mucosa and the absence of invasion of the underlying tissue, it cannot be recognized in smears. One may hazard a guess that smears containing cells resembling metaplastic ciliated or mucous epithelium might indicate this early and non-invasive form, but that is all.

Bronchial (or Tracheal) Adenoma. No definite criteria can be advanced for the recognition of the bronchial adenoma. The presence of small, overstained cells with dark nuclei and *granular* cytoplasm might indicate its presence. The fact that the two examples in this series were misdiagnosed as anaplastic carcinomas points the moral.

Alveolar or Terminal Bronchiolar Carcinoma. Theoretically speaking, alveolar cell carcinoma should be recognizable in smears because its cells are pleomorphic and apt to form bizarre clusters and to show vacuolization; practically stated, however, these neoplasms are usually confused with either pleomorphic carcinomas or adenocarcinomas and diagnosis of the type is therefore unreliable. None of the 5 instances in the series was correctly diagnosed.

Sarcoma. The only sarcoma in the series was characterized by abundant fusiform fibroblasts which were strikingly prominent in the smears. The cells were metaplastic, overstained, and unrecognized as sarcomatous. It was diagnosed as metastatic pleomorphic tumor.

Malignant Teratoma. As malignant teratoma is of very mixed structure, the diagnosis will depend on which of its components exfoliate and appear in smears. In the lone example in the series, only the epithelial elements exfoliated and the diagnosis was epidermoid carcinoma, which was manifestly erroneous.

TABLE V

Data on Miscellaneous Pulmonary Tumors

Incidence in women		Incidence in total series		Diagnostic accuracy as to type		Laboratory diagnosis		Writer's diagnosis	
	per cent		*per cent*		*per cent*				
N. Y. H.*	100.0	N. Y. H.*	0.7	N. Y. H.*	0.0	S*		S*	
M. H.	100.0	M. H.	0.5	M. H.	0.0	G	2	G	
All	100.0	All	0.58	All	0.0	W		W	2

* N. Y. H., New York Hospital; M. H., Memorial Hospital. S, specific; G, general; W, erroneous diagnoses, respectively.

TABLE VI

Data on Alveolar-Celled Carcinoma

Incidence in women		Incidence in total series		Diagnostic accuracy as to type		Laboratory diagnosis		Writer's diagnosis	
	per cent		*per cent*		*per cent*				
N. Y. H.*	50.0	N. Y. H.*	1.41	N. Y. H.*	0.0	S*		S*	
M. H.	0.0	M. H.	1.5	M. H.	0.0	G	5	G	
All	0.3	All	1.46	All	0.0	W		W	5

* N. Y. H., New York Hospital; M. H., Memorial Hospital. S, specific; G, general; W, erroneous diagnoses, respectively.

TABLE VII

Data on Other Tumors

Incidence in women		Incidence in total series		Diagnostic accuracy as to type		Laboratory diagnosis		Writer's diagnosis	
	per cent		*per cent*		*per cent*				
N. Y. H.*	100.0	N. Y. H.*	1.42	N. Y. H.*	0.0	S*		S*	
M. H.		M. H.	0.0	M. H.		G	2	G	
All	100.0	All	0.59	All	0.0	W		W	2

* N. Y. H., New York Hospital; M. H., Memorial Hospital. S, specific; G, general; W, erroneous diagnoses, respectively.

DISCUSSION

It is evident from the foregoing descriptions that the type as well as the presence of pulmonary cancer can be diagnosed with satisfactory accuracy in smears of sputum and/or bronchial aspirates. It will be noted that the degree of accuracy of this diagnosis was greater in the series from the New York Hospital than in that from Memorial

Hospital. That this is entirely attributable to the purely objective method employed in collecting the diagnoses in the latter series is unlikely, as the data available in the more subjectively studied group of smears from New York Hospital were usually of a general nature and the laboratory diagnoses as often unspecific. The smears from Memorial Hospital were much more difficult to evaluate; they showed fewer outstandingly characteristic pictures than did those of the other series. It is not improbable that the large number of smears from bronchial aspirates (and more particularly from nasotracheal aspirates), in proportion to those of sputum (more numerous in the New York Hospital sets), may have played a part in rendering evaluation more difficult. Smears of bronchial aspirates always present greater numbers of isolated and scattered neoplastic cells and fewer clusters, so important in diagnosing types of tumor, so that their examination entails the exercise of a great deal more judgment and application. This has already been mentioned in connection with the anaplastic carcinomas, the detection of which is harder in bronchial aspirates containing many distorted but normal epithelial cells from the bronchial lining.

During 1951 McDonald and his co-workers[2] published a paper on the significance of cell types in bronchogenic carcinoma, their material comprising 849 cases that withstood rigid scrutiny as to the requirements for a diagnosis of primary bronchogenic carcinoma. Each type is discussed by a separate group of investigators who have examined the cases from both clinical and pathologic standpoints. They found that the accuracy of cytologic diagnosis as to the presence of pulmonary carcinoma was about 90 per cent, which is the figure generally maintaining in our laboratory and in others throughout the country. A comparison of their figures on percentages of occurrence of the various types and of female patients involved with similar figures obtained in the present investigation follows.

In Table VIII percentages of the various types in the present investigation are figured on a total of 327 tumors, while the percentages

TABLE VIII

Comparison of Data Obtained by McDonald et al.[2] and in This Investigation

Type	Relative total incidence		Incidence in females	
	McDonald *et al.*	This investigation	McDonald *et al.*	This investigation
	per cent	*per cent*	*per cent*	*per cent*
Epidermoid carcinoma	37.8	56.0	3.5	3.4
Pleomorphic carcinoma	40.2	20.0	14.6	13.9
Adenocarcinoma	13.2	11.5	20.0	10.7
Anaplastic carcinoma	8.8	12.5	3.3	7.3

in Tables I to VII were reckoned on a total of 341 tumors in the entire series of pulmonary growths. This accounts for the slight differences. Metastatic growths and esophageal tumors totaled 15, and these were eliminated from the count in Table VII. The most marked differences between the percentages obtained in this investigation and those arrived at by McDonald and his colleagues[2] are to be found in the epidermoid and pleomorphic categories; obviously this is the result of differences in interpretation. There is reasonably close agreement between the two sets of figures concerning the sex incidence of these tumors.

I have made a further check on the percentage of incidence of the various types of pulmonary cancer by referring to the copious material in the Registry of Thoracic Tumors in the Armed Forces Institute of Pathology in Washington. First, 123 cases were reviewed by checking the diagnoses under the microscope; it was found that there was better than 90 per cent agreement between the diagnoses coded by the Institute and my opinion after inspecting the sections. The percentage of incidence in this series is shown in Table IX. This distribution

TABLE IX

Incidence as to Type in 123 Cases of Pulmonary Cancer at the Armed Forces Institute of Pathology

Type	Number	Percentage	Type	Number	Percentage
Epidermoid	55	45	Alveolar	5	4
Pleomorphic	15	12	Bronchial adenoma	1	1
Adenocarcinoma	14	11	Others	5	4
Anaplastic	28	23	Total	123	100

shows fewer epidermoid carcinomas and almost twice as many anaplastic carcinomas as were present in the New York series. It should be remembered that while the Institute series represents a broad range of ages, it is heavily weighted with cases involving patients of military age. This would be expected to increase the percentage of anaplastic carcinomas which are most frequently encountered in the fourth decade.

An additional 206 cases were next reviewed without checking the diagnoses microscopically, since the agreement had been so excellent in the first 123 cases. However, many of these diagnoses had not been revised at the Institute, but had been made in hospitals (mostly military) throughout the country. As almost no pleomorphic carcinomas were listed in this series, the microscopic descriptions were consulted and it was found that several pleomorphic carcinomas had been in-

cluded under the diagnosis of adenocarcinoma, from which category they were accordingly transferred to the pleomorphic group. The distribution in this series was then as shown in Table X.

TABLE X

Incidence as to Type in an Additional 206 Cases of Pulmonary Cancer at the Armed Forces Institute of Pathology

Type	Number	Percentage	Type	Number	Percentage
Epidermoid	115	55.8	Alveolar	1	0.5
Pleomorphic	18	8.9	Bronchial adenoma	0	0.0
Adenocarcinoma	20	9.7	Others	3	1.5
Anaplastic	49	23.5	Total	206	100.0

Combining the findings of these two series the results are as shown in Tables XI and XII. It will be seen that, on the whole, agreement is

TABLE XI

Combined Figures on Incidence and Type for the Two Series from the Armed Forces Institute of Pathology

Type	Number	Percentage	Type	Number	Percentage
Epidermoid	170	51.5	Alveolar	6	1.8
Pleomorphic	33	10.0	Bronchial adenoma	1	0.6
Adenocarcinoma	34	10.3	Others	8	2.4
Anaplastic	77	23.4	Total	329	100.0

TABLE XII

For Comparison with the Figures of Table XI, the New York Cases Are Tabulated in a Similar Manner*

Type	Number	Percentage	Type	Number	Percentage
Epidermoid	175	53.5	Alveolar	5	1.5
Pleomorphic	65	19.8	Bronchial adenoma	2	0.7
Adenocarcinoma	37	11.3	Others	2	0.7
Anaplastic	41	12.5	Total	327	100.0

* In tabulating the New York cases, 14 have been rejected: 8 esophageal and 6 secondary adenocarcinomas, which make up the difference between 341 and 327.

fairly close. There are more anaplastic carcinomas in the A.F.I.P. series, while there are proportionately more pleomorphic carcinomas in the New York Hospital series; even so, the percentage of pleomorphic carcinomas in the latter lags far behind that noted in the Mayo Clinic tables.

SUMMARY AND CONCLUSIONS

An investigation of 341 sets of smears of sputum and/or bronchial aspirates indicates that the type, as well as the presence of pulmonary

cancer, can be determined by cytologic examination with a degree of accuracy of 80 per cent.

In the series there were 183 epidermoid carcinomas, 8 of them of esophageal origin; 65 pleomorphic carcinomas; 43 adenocarcinomas, 6 of them secondary to tumors of other organs; 41 anaplastic carcinomas (oat-cell); 5 alveolar or terminal bronchiolar carcinomas; 2 bronchial adenomas; and one example each of malignant teratoma, and fibrosarcoma.

Expressed in percentages of incidence, the representation of the various types of primary pulmonary cancer was as follows: epidermoid carcinoma, 53.5; pleomorphic, 20.2; adenocarcinoma, 11.1; anaplastic or oat-cell carcinoma, 12.5; bronchial adenoma, 0.6; alveolar or terminal bronchiolar carcinoma, 1.5; and fibrosarcoma and malignant teratoma, 0.3 per cent each.

The percentages of accuracy of diagnosis for the various types were: epidermoid carcinoma, 89.9; pleomorphic carcinoma, 69.2; adenocarcinoma, 81.4 for all types, 78.4 for primary growths; anaplastic carcinoma, 83.5; and all other types, 0.

Smears of sputum have been found to present better evidence for the recognition of the various types than do those of bronchial aspirates.

Access to the records and microscopic sections of Memorial Hospital was afforded by its pathologist, Dr. F. W. Stewart, whose friendly cooperation is much appreciated.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 149

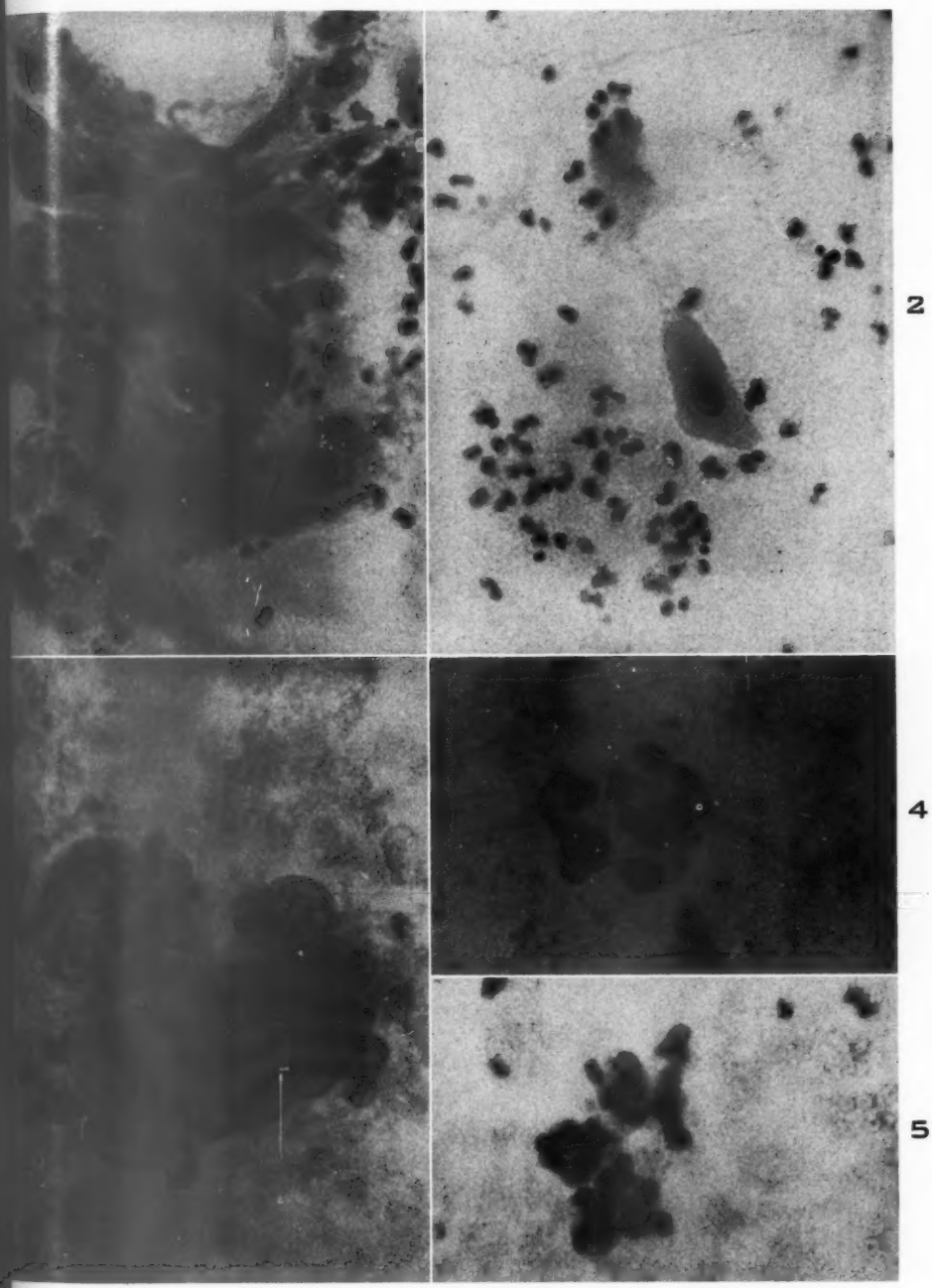
FIG. 1. Large fragment of epidermoid carcinoma in sputum; it is composed of elongated, interlacing and heavily keratinized cells which typify the group. It is unusual to discover such large clusters in smears. × 600.

FIG. 2. Single, characteristically keratinized cell from epidermoid carcinoma, triangular in outline and with a hyperchromatic nucleus. Its cytoplasm was intensely acidophilic. × 600.

FIG. 3. Fragment of pleomorphic carcinoma comprising many frankly pleomorphic cells which are unkeratinized and *rounded,* rather than triangular or elongated. For comparison with Figure 9, which bears a striking similarity. × 600.

FIG. 4. Cluster of cells from a primary adenocarcinoma. They have delicate vacuolated cytoplasm and hyperchromatic nuclei. In this case they are concentrically arranged. × 600.

FIG. 5. Typical tightly organized cluster of tiny cells with very hyperchromatic nuclei in which there is little detail. From bronchial aspirate from case of anaplastic or oat cell carcinoma. × 600.



Foot Pulmonary Cancer in Cytologic Smears

PLATE 150

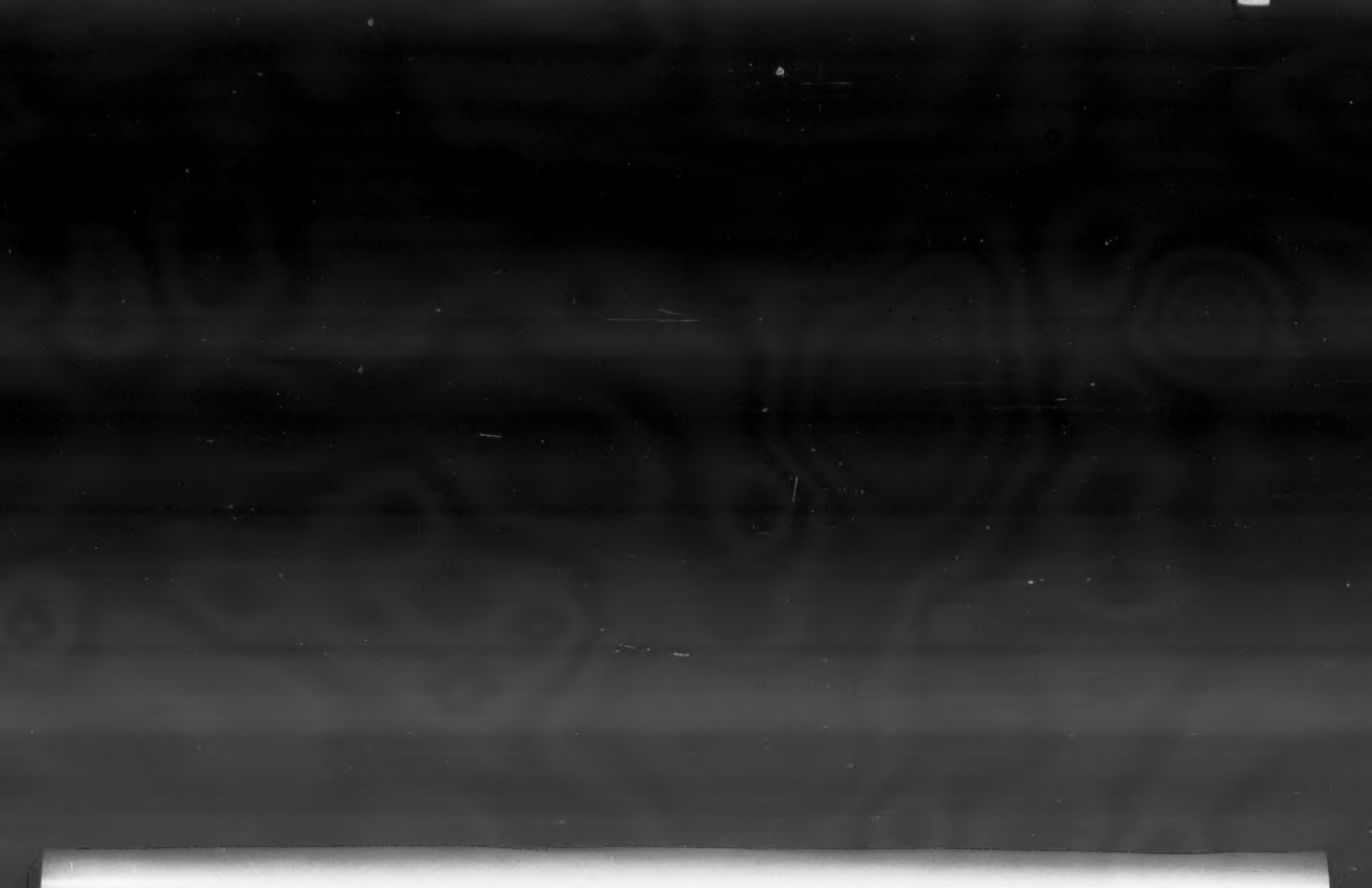
FIG. 6. A strip of cells from a primary adenocarcinoma; they appear to have "lined something" before becoming detached and exfoliating. × 600.

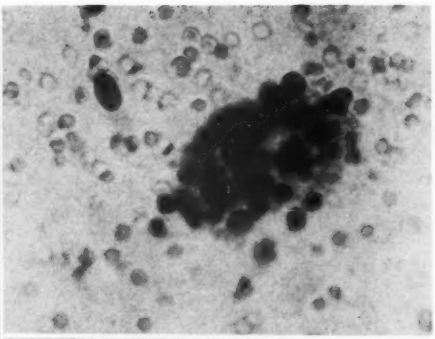
FIG. 7. Typical tightly organized cluster of tiny cells with very hyperchromatic nuclei in which there is little detail. From bronchial aspirate from case of anaplastic or oat cell carcinoma. × 600.

FIG. 8. A triangular cluster of small cells from a bronchial adenoma. Such structures can be readily confused with those of anaplastic carcinoma. This was the mistake in this particular case. On closer scrutiny it will be noted that the nuclei present more detail and possess nucleoli. The granular cytoplasm usually seen in sections is not evident in the smear. × 600.

FIG. 9. Highly pleomorphic and metaplastic cells from an alveolar cell carcinoma; recovered in sputum. Their similarity to those of the pleomorphic carcinoma shown in Figure 3 is obvious and might condone a mistaken diagnosis in this case of "pleomorphic carcinoma." × 600.

FIG. 10. A section from the lung of the patient from which the smears for Figure 9 were obtained. The identity of the appearance of the cells in the section and the smear is manifest. × 600.





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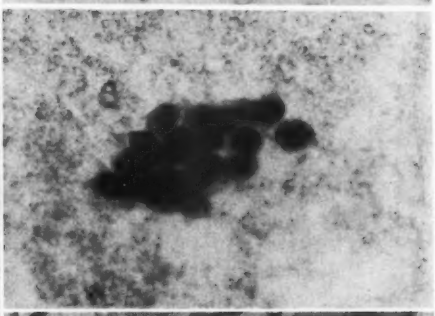

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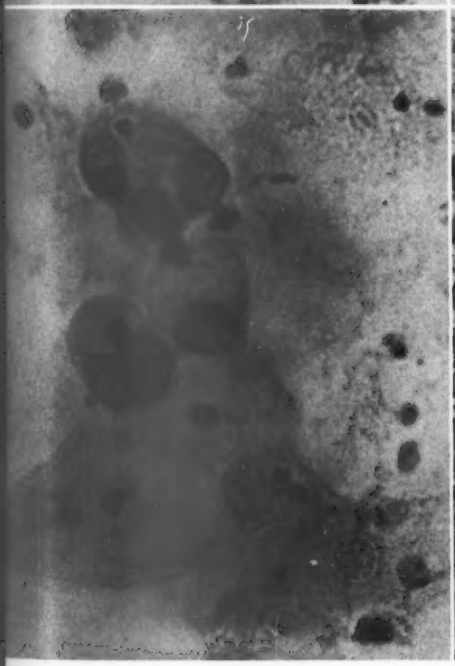

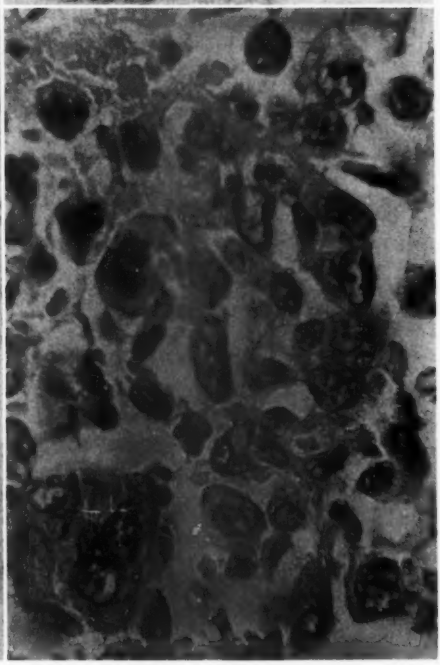

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Foot

Pulmonary Cancer in Cytologic Smears

Plate 151

Fig. 11. A lower magnification of a smear containing innumerable clusters of cells quite foreign to the lung; the patient had an adenocarcinoma elsewhere in the body, probably the pancreas. It is very unlikely that primary adenocarcinoma would ever present smears with as many clusters as there are in this one. × 60.

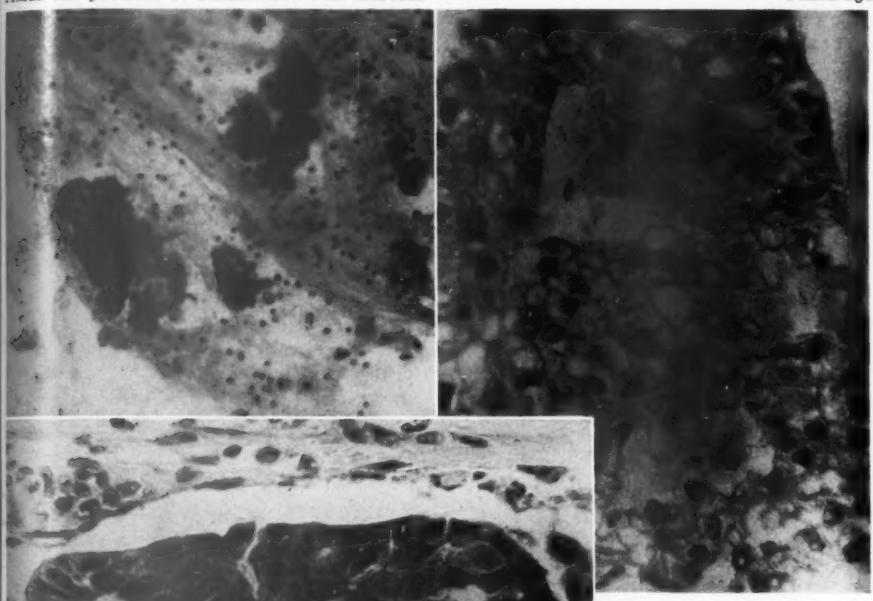
The photomicrographs for Figures 12 to 15 were prepared from tissue sections.

Fig. 12. Pleomorphic type; in this tumor the cells are fairly uniform and the growth could be called large celled or, in view of its somewhat adenoid aspect, adenocarcinoma. × 305.

Fig. 13. Typical adenocarcinoma primary in parabronchial glands. × 305.

Fig. 14. Epidermoid bronchogenic carcinoma, from a section of one of the tumors in the series from New York Hospital. × 305.

Fig. 15. Typical anaplastic, undifferentiated, small celled, or oat celled carcinoma. × 305.



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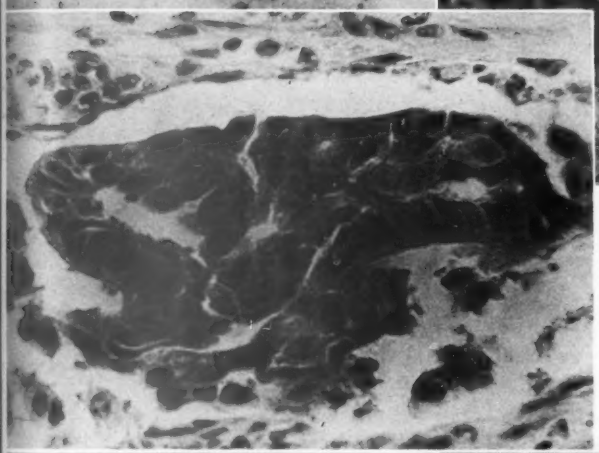

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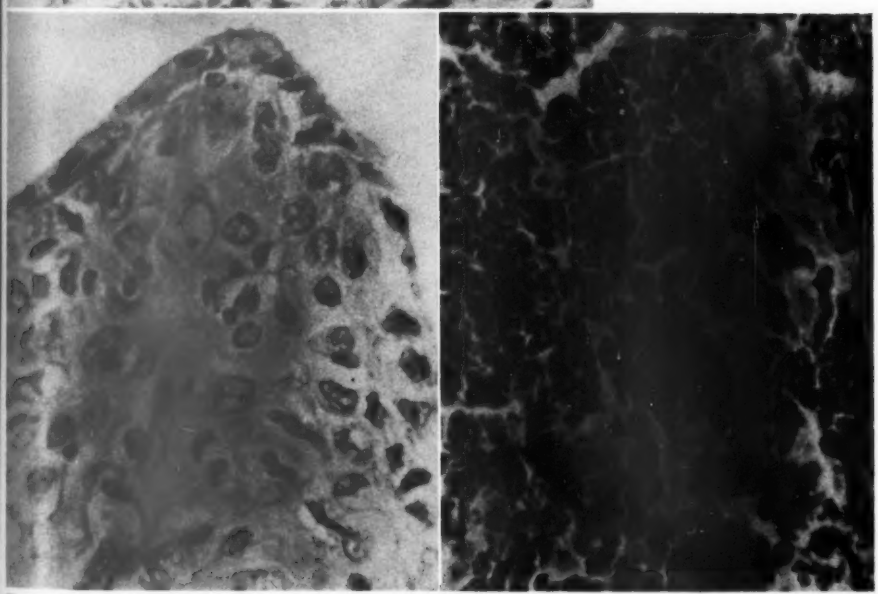

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Pulmonary Cancer in Cytologic Smears

THE CYTOLOGY OF SKIN PAPILLOMAS THAT YIELD VIRUS-LIKE PARTICLES *

HENRY BUNTING, M.D., MAURICE J. STRAUSS, M.D., and WILLIAM G. BANFIELD, M.D.
(From the Department of Pathology and the Division of Dermatology, Yale University School of Medicine, New Haven, Conn.)

Certain warts from the hands and feet have been found to differ clinically from other warts from the same sites in that they have a smooth margin, relatively less keratinization, and a surrounding erythematous inflammatory halo. Warts of this type alone yielded, in their suspensions, spherical virus-like particles under the electron microscope.[1] They also possessed intranuclear inclusion bodies and characteristic cytoplasmic masses which the others lacked. Lyell and Miles,[2] in England, independently recognized the two clinical groups and observed the cellular differences. Lyell[3] has confirmed the presence of the virus-like particles in suspensions of the warts with the intranuclear inclusion bodies and cytoplasmic masses. The present report is a description of the cytologic features of the lesions of the type which yield virus-like particles.

The effect of viruses on cells may apparently be characterized as either necrosis or stimulation. Warts, having long been known to be transmissible directly or by their filtrate,[4-7] have been regarded as being caused by a virus. The effect of the agent causing warts would appear to be chiefly stimulation with resultant papillomatous overgrowth of the epidermis. In the warts that have yielded virus-like particles a further effect which is apparently the result of the presence of the virus is to be emphasized; this is a distortion in the orderly development and differentiation of the epidermal cells. Such distortion is absent in warts of the type from which virus-like particles have not been obtained.

MATERIALS AND METHODS

The papillomas used for this study were obtained from the clinical practice of one of us (M. J. S.). The material has been reported upon previously; the incidence and other clinical features of the lesions have been presented.[8] A brief description of the histologic characteristics has been given.[9]

In the present studies the excised wart was cut in two and one por-

* Supported by a grant from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

Presented in part at the Forty-eighth Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, April 28, 1951.

Received for publication, May 9, 1952.

tion of the tissue was fixed in 10 per cent buffered (pH 7) formalin unless otherwise noted. Sections were stained with Harris's hematoxylin and eosin. Basophilia was detected by staining in dilute methylene blue (concentration, 5×10^{-4}M) buffered at different pH's (solutions were prepared after the method of Dempsey and Singer[10]); acidophilia was determined by staining in dilute aniline blue (concentration, 5×10^{-4}M) buffered at different pH's. Ribonucleic acid distribution was determined by digestion in crystalline ribonuclease in distilled water at a pH of approximately 6.3 (proteolytic activity had been removed by the method of McDonald.[11] The digestion was conducted at 56° to 59° C. for 2 hours. The slide, so treated, and a duplicate section incubated under similar conditions in distilled water were stained in dilute methylene blue (concentration, 5×10^{-4}M at pH 7.6) for 1½ hours, then air dried, placed in xylol, and mounted in a synthetic mounting medium (harleco). Control ribonucleic acid-containing material, the basophilic bodies of the hepatic cells, was always run along with the experimental material. The desoxyribonucleic acid was determined by the Feulgen reaction.[12,13] Lipid was detected by staining in Sudan black B in glycol, employing a longer exposure than usual (1 to 2 hours)[14]; osmic acid also was employed on unfixed tissue (a) without subsequent exposure to alcohol and (b) in the chlorate-formalin method of Swank and Davenport.[15] While it is realized that osmic acid is not specific in its reaction with lipids, it is believed that it lends support to the evidence derived by other methods.[16] Microincineration was done in a small muffle furnace with a temperature of 550° to 600° C. maintained for 30 minutes or more. The coverglass was then sealed with paraffin and the ash examined by dark field illumination. The illuminating source was a 6 volt incandescent tungsten bulb burned at approximately 8 volts to reduce the amount of yellow in the light. This is necessary since a yellow color of the ash is to be regarded as evidence of the presence of iron oxide.[17]

Oxidation of the section in chromic acid (Bauer technic of Lison[12]) before exposure to Schiff's reagent was carried out on tissue fixed in Rossman's fluid. For the identification of glycogen one section was first incubated in malt diastase (0.2 per cent solution buffered at pH 5, for 2 hours at 37° C.) and compared with a control, incubated similarly in buffer before chromic acid treatment. Periodic acid oxidation[18] also was used at times for the detection of glycogen. We have been uncertain concerning the interpretation of areas in sections that are stained with Schiff's reagent after periodate unless they were further identifiable by their susceptibility to the action of certain enzymes.[19] If such susceptibility is absent, it will merely be stated

that a positive reaction has been obtained and it will be assumed that this was due to the existence of the proper arrangement of the required groups[20,21] without conjecture as to the class of compound this may indicate.

In a previous report[8] the correlation was presented between finding a characteristic cytologic picture and virus-like particles in the same wart. That material has been augmented and the full data of this part of the study are presented here.

Results

Twenty-nine plantar and common warts were examined by both histologic section and electron microscopy of their suspensions in distilled water. Seventeen had neither intranuclear inclusions nor cytoplasmic masses as will be described; their suspensions did not yield virus-like particles by electron microscopy. All nine that showed intranuclear inclusion bodies and characteristic cytoplasmic masses yielded virus-like particles in the suspensions made from a portion of the lesion. Three yielded particles but the intranuclear and cytoplasmic bodies could not be found in the portion of the wart taken for histologic study. The most likely explanation of this discrepancy is suggested by an occasional instance wherein only two or three papillary processes in the portion sectioned for light microscopy contained the characteristic findings, while the remainder showed only acanthosis and hyperkeratosis. It seems reasonable that in the few in which this correspondence was not found the fragment saved for histologic examination by chance did not contain any papillary processes with the cellular changes. One may conclude that the intranuclear inclusion bodies and the cytoplasmic masses are the result of the presence of the virus—if the particles do represent such. Certainly the deviation from the normal development of the epidermal cells encountered in these lesions appears to be associated in some manner with the presence of the virus. The differentiation of the constituent cells of the warts from which particles have not been obtained deviates somewhat from normal but not to the degree encountered in the lesions to be discussed. It is these latter changes that will be described in detail.

The eosinophilic, intranuclear inclusion bodies appear first in cells low in the malpighian stratum, not in those of the basal layer, but in those of the first or second layer above the basal (Figs. 1 and 2). The inclusion body is small here, averaging 1.6 μ in diameter; the smallest measured was 1.0 μ in diameter. It increases in size progressively to an average of 3.2 μ in the cells of the lower stratum corneum (Figs. 3, 5, and 6). (Fifty-seven inclusion bodies were measured in making

these determinations.) This tissue had been subjected to the shrinkage of formalin fixation and paraffin imbedding. The body is surrounded by a clear halo (Fig. 4). It is less basophilic and somewhat more acidophilic than the chromatin as shown by staining in buffered solutions of methylene blue and aniline blue respectively, as will be described. The inclusion body is Feulgen negative in contrast to the chromatin masses. In this determination the identification of the inclusion body was assured by visualizing the same cell stained with hematoxylin and eosin after de-staining the Feulgen preparation (Figs. 9 and 10). Colored photomicrographs were made of the same field stained with both procedures for absolute certainty of the results. The morphologic differentiation between the inclusion body and the nucleolus also appears to be clear. As noted, inclusion bodies are not seen below the second or third layer of cells of the malpighian stratum, while nucleoli are identifiable in the basal layer as well as in the cells above. In addition, the line of demarcation between the region of the wart which contains intranuclear inclusion bodies and other characteristic changes and that at the periphery where there is only hyperplasia and hyperkeratosis is distinct. Nucleoli are common in these latter hyperplastic epidermal cells, but no intranuclear inclusion bodies (or cytoplasmic masses) have been found. Further, in many instances both a nucleolus and an inclusion body are present and clearly distinguishable from each other within the same nucleus. Ribonuclease does not appreciably diminish the basophilia of the inclusion body or of the cytoplasmic masses, while it reduces greatly the general basophilia of the cytoplasm of the epidermal cells. Finally, photomicrographs* taken at 2650 Å show no selective absorption by the inclusion body in contrast to that exhibited by the nuclear membrane and chromatin granules. At 2800 Å the difference between the absorption by the nuclear structures and by the inclusion bodies (or by the cytoplasmic masses) is relatively less than at 2650 Å (Figs. 11 and 12). It is concluded from these data that the eosinophilic intranuclear inclusion body is distinct from the nucleolus and further that neither desoxyribose nor ribose nucleic acids can be detected in it by the methods employed.

In a few instances in which the staining intensity was optimal, it has been possible to follow the intranuclear inclusion body from the stratum Malpighii into the stratum corneum. The nuclei of the cells in the

* We are grateful to Dr. Jesse F. Scott of the Department of Biology, Massachusetts Institute of Technology, and the Huntington Laboratory, Massachusetts General Hospital, for the ultraviolet photomicrography.

lower portion of the stratum corneum are only about one-half the size of those in the uppermost part of the stratum Malpighii, and have become almost round; the intranuclear inclusion body has become much larger, filling the nucleus but for a thin rim surrounding it in which chromatin material is sometimes visible.

While a high proportion of cells may show the characteristic changes, there are usually a few cells unaffected in any area (Figs. 6 and 7). The affected cells are considerably larger than the normal cells. Mitotic figures have not been observed in them while they are fairly common among the other cells of comparable levels of the stratum Malpighii. The nuclei containing the inclusion bodies also show abnormalities. They are larger and have more chromatin stainable by hematoxylin, basic dyes, or the Feulgen technic; and, in addition, may be irregular in shape or rarely may be multiple, with two or three present. As noted, the nuclei persist as deeply staining (by the methods used), rounded, or somewhat shrunken structures in the lower stratum corneum above that portion of the wart showing the characteristic changes. In contrast, the stratum corneum overlying the merely hyperplastic and hyperkeratotic margins or the stratum corneum of common warts from which the virus-like particles were not recovered did not show parakeratosis. As in other epidermal lesions parakeratosis here was accompanied also by the cytoplasmic abnormality of absence of the stratum granulosum; no keratohyaline granules were present in the cells containing inclusion bodies, although they were constantly observed in occasional normal cells among them.

Further evidence of developmental abnormality is given by the interference in formation of intercellular bridges (Fig. 8) by the affected cells. They are usually absent between two adjacent cells when both are affected. They are present, however, between two cells when only one is affected, leading to the conclusion that in this instance the bridges probably are the product of the unaltered cell.

The stratum corneum overlying that portion of the papilloma with the cytologic changes which have been described often differs from that of simple hyperkeratosis in that its appearance on section may be that of an open net-like pattern giving the impression of strands of keratin at the periphery of the cells surrounding an empty space. This is particularly true in the deeper layers while the more superficial layers of the stratum corneum are often somewhat more compact.

Numerous small bodies are present in the cytoplasm of the cells low in the stratum Malpighii which contain intranuclear inclusions. They exhibit about the same degree of basophilia and acidophilia as do the

intranuclear inclusions. This has been investigated by determining the pH of the staining solution on either side of neutrality beyond which the particular structures adsorb no dye. The staining of the nuclear chromatin has been used in all instances as the reference for comparison. Since it is known that the degree of basophilia of tissue is modified by the effect of the particular fixative,[22] the results in sections fixed in formaldehyde buffered at neutrality and also in Helly's fluid have been determined.

Both the inclusion body and cytoplasmic masses lost their affinity for methylene blue at a pH of 5.7 when fixed in Helly's fluid and at a pH of 4.6 after fixation in 10 per cent formalin buffered at neutrality. Nuclei stained well at both these pH values, losing their ability to stain after Helly's fluid at a pH of 4.6 and at a pH lower than 3.6 after formalin. The pyknotic nuclei of the stratum corneum showed a very much greater affinity for methylene blue at low pH levels than did the other nuclei. From these observations one would conclude that the acidic groups present in the intranuclear inclusion bodies and cytoplasmic masses were weaker acids than those of the nuclear structures. Incubation of the section in ribonuclease was without apparent effect upon the basophilia of either of these structures. It should be emphasized that the basophilia of the cytoplasm between the granules, which was moderate in amount, was removed by the enzyme. This was true also of the cytoplasmic basophilia of the lower layers of the adjacent epidermis, as might be expected. As noted earlier, these cytoplasmic masses do not absorb ultraviolet light selectively at a wave length of 2650 Å, offering further evidence of the absence of nucleic acids as a significant part of their structure.

The acidophilia of the inclusion bodies or cytoplasmic masses as shown by their affinity for an acidic dye, aniline blue, was somewhat greater than that of the nuclear membrane or chromatin, the former staining at a slightly higher pH (6.8) than the latter (6.3). The acidophilia of the cytoplasmic masses when they were first formed was only slightly less than that of the stratum corneum; as the masses increased in size in the upper malpighian layers their acidophilia became about equal to that of the overlying keratinized stratum corneum.

Resuming the detailed description of the cytoplasmic masses, it can be observed that when they first appear they are small and multiple; about 10 or 20 are present in each cell. They increase in size as the cells mature in the higher levels of the malpighian stratum, but become fewer in number and acquire a rounded vacuole which grows with the granule. Finally, in the layers beneath the stratum corneum, these

bodies lose their vacuoles and fuse into one, two, or three larger masses which at times occupy almost the entire cytoplasm. After prolonged exposure to Sudan black B in glycol, these cytoplasmic masses acquire a faint staining, giving indication of lipids within them. Osmic acid employed by both methods described (without subsequent exposure to alcohol in either) has stained the cytoplasmic masses faintly brown. Further evidence of the presence of a lipid is afforded by the growth of the vacuoles in the cytoplasmic masses, as observed in the fresh state, when the tissue is allowed to remain in dilute alcohol (25 to 50 per cent solution in distilled water at room temperature) before being placed in formaldehyde. Under these conditions the vacuole in each cytoplasmic mass increases greatly in size, but is constantly surrounded by a thin rim composed of the distended cytoplasmic mass; the enlarged vacuole at times develops an irregular but rounded contour. This process is interpreted as the formation of "myelin figures" (Fig. 13). While lecithin is the lipid classically described as giving rise to these structures, it is known that others may do so also.

No birefringence of the masses has been observed when they were viewed between crossed Nicol prisms, giving no evidence, therefore, of a preferential orientation of the submicroscopic constituents.

After microincineration the dark-field examination reveals that there is a greater amount of ash derived from the affected cells than from the others. The intranuclear inclusion body has not, however, been found to form a recognizable deposit of ash. The ash from the nuclear membrane and chromatin in the affected cells is much heavier and is a faint yellow in contrast to the blue-white nuclear ash of the unaffected cells. That from the cytoplasmic masses is equally conspicuous and of the same yellow color. The yellow color is interpreted to indicate the presence of iron in these structures.[17]

The presence of glycogen was sought in the cells of the papillomas by the use of periodic and also chromic acid oxidation prior to applying Schiff's reagent, controlled by previous maltase digestion. None was found in the vacuoles of the cytoplasmic masses and it was found only infrequently in the cytoplasm of the affected cells as a few fine granules. This is in contrast to the moderate amounts diffusely demonstrable through the cytoplasm of the unaffected cells of the papilloma which were comparable with those usually found in the cells of the stratum spinosum of many hyperplastic and neoplastic epidermal lesions (unpublished results). Periodic acid oxidation followed by Schiff's reagent brought out faintly the intercellular bridges between the unaffected cells and occasional areas in the keratin overlying both

the affected and unaffected zones. The intranuclear inclusion bodies and cytoplasmic masses and vacuoles were consistently negative to Schiff's reagent after periodic acid oxidation.

Discussion

In the foregoing there have been described the cytologic features of certain papillomas of the skin which yield virus-like particles in their suspensions. These features are not found in warts from which the virus-like particles have not been obtained and it therefore has been concluded that these particular changes result in some way from the presence of the virus in the cells. There is interference in the normal development of the nucleus and differentiation of the cytoplasm. A round eosinophilic body appears within the nucleus of the cells low in the stratum Malpighii. This enlarges progressively as the cells of the papilloma mature. It is Feulgen negative and hence is not chromatin. The body has been considered to be an intranuclear inclusion body, probably related to the presence of the virus, rather than a nucleolus for the following reasons: it is present only in those cells of the papilloma that possess the other constant features observed in this type of papilloma, and not, for example, in the cells of the immediately adjacent epidermis that shows only acanthosis and hyperkeratosis or in the occasional normal cell in the midst of the abnormal ones; contrary to structures that are rich in nucleic acid, it does not selectively absorb ultraviolet light of wave length 2650 Å as opposed to 2800 Å; the body is commonly seen in nuclei that contain also a structure showing a strong affinity for hematoxylin and typical of a nucleolus. This inclusion body appears to be of the type designated as B in Cowdry's classification[23] inasmuch as the inclusion is eosinophilic, is circumscribed, and without margination of the chromatin. Further evidence of the distinction between the intranuclear inclusion body and a nucleolus is afforded by the final development of the inclusion body. In the cells of the lower stratum corneum the inclusion body enlarges greatly, almost entirely filling the nucleus.

Cells with intranuclear inclusion bodies also possess other characteristics not found in the adjacent hyperplastic and hyperkeratotic epidermis or in warts that do not yield the virus-like particles. There apparently is an interference in cell division. Mitotic figures have not been observed in the affected cells. Probably as a consequence of this, the nucleus and the entire cell are considerably larger than normal. This explanation appears much more likely than that polyploidy exists in all the affected cells. The nuclei increase in size continuously as the cells mature, which is consistent with progressive growth uninterrupted

by cell division. Further, the nuclear membrane in these cells is heavier and more irregular than normal and yields a much greater amount of ash. This ash appears to contain iron.

Warts having the cytologic characteristics described here are the only ones in our studies that have yielded virus-like particles. The warts studied by Hydén[24] do not have these cytologic features. He described and illustrated ultraviolet absorbing material (2650 Å) in the nucleus which first appears around the nucleolus, increases in amount, and finally occupies the entire nucleus in cells that one would interpret from the photomicrographs as of the lower stratum corneum. These warts, as well as those studied by Blank, Buerk, and Weidman,[25] have had inclusion bodies of the Lipschütz type. Blank and co-workers have followed Hydén's thesis in discussing the features of the warts they investigated. The interpretation of their data in relation to the papillomas under consideration here is difficult, however, since only one of their several illustrations pictures a wart having unmistakably this cytologic appearance (*cf.* Fig. 5, page 24 of their article). The structures indicated by arrows and designated as "large nucleoli" we believe to be inclusion bodies; and it is the development of these which has been followed in the present study, from their first appearance low in the stratum Malpighii to their maximum growth in the stratum corneum. We concur with the conclusion of Blank, Buerk, and Weidman, which can be applied also to the warts under consideration, that the nucleus finally is filled with inclusion material. The intranuclear inclusion bodies that Lipschütz described in common warts have been interpreted by van Rooyen and Rhodes[26] as "probably of degenerate nature."

The cytoplasmic granules in the affected cells do not appear to contain ribonucleic acid but react as if they had a lipid component. The granules stain faintly with Sudan black B and osmic acid, and under the proper conditions appear to form "myelin figures." It is realized that osmic acid does not react specifically with lipids. In this instance, however, the reaction confirms the others. The relationship of these to keratohyaline granules, which are absent in the cells of the papilloma, cannot be stated since keratohyalin has not been definitely characterized histochemically. It is observable that hematoxylin has a much greater affinity for keratohyalin in the normal epidermis than for the granules in question. The true significance of both the intranuclear and cytoplasmic bodies is at present being sought by use of the electron microscope.

The interference in formation of intercellular bridges by the affected cells apparently would indicate a disturbance in cytoplasmic differ-

entiation of the squamous epithelium. The absence of keratohyaline granules in the cells of the papilloma is associated, as would be expected, with persistence of nuclei in the stratum corneum (parakeratosis). The imperfect cornification of the overlying layers also is not surprising. It should be noted that a disturbance of this type differs from the vacuoles in the cells of the stratum Malpighii in epidermodysplasia verruciformis.[27,28]

Summary

Plantar and vulgar warts from which virus-like particles have been obtained are distinguishable by certain cytologic characteristics. There are eosinophilic intranuclear inclusion bodies and vacuolated cytoplasmic masses in the cells of these papillomas that are absent in the warts from which the particles have not been found, in spite of the similarity of the papillomatous structure in both. Nuclear enlargement and distortion, absence of mitotic figures, and interference in development of the epidermal cells probably all reflect the effect of the virus.

We wish to express our appreciation for the valuable technical assistance of Miss Patricia M. Reynolds.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 152

FIG. 1. The warts that yield virus-like particles have a typical papillomatous form and hence resemble those that do not yield elementary bodies. Heavy keratinized layers are on top, and rete pegs can be seen at the bottom. Hematoxylin and eosin stain. × 15.

FIG. 2. Lower layers of the stratum Malpighii and underlying papilla of wart that yields virus-like particles. The intranuclear inclusion bodies and characteristic cytoplasmic masses are evident in cells of all but the basal-most two layers of the stratum Malpighii. In the midst of the others there is an unaffected cell with clear cytoplasm and no intranuclear inclusion body. Its nucleus is much smaller than those of the other cells. Hematoxylin and eosin stain. × 485.

FIG. 3. Base of wart at lower right. Nucleoli as well as intranuclear inclusion bodies are present in many cells; the latter can be seen to increase in size as the cells mature (toward the upper left). Hematoxylin and eosin stain. × 800.

VIRGIN

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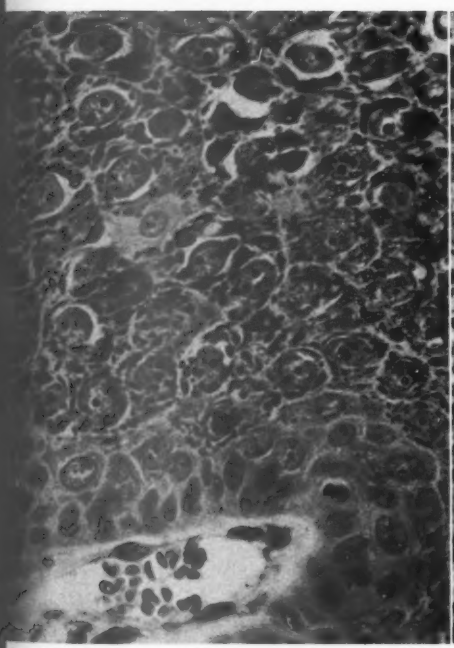

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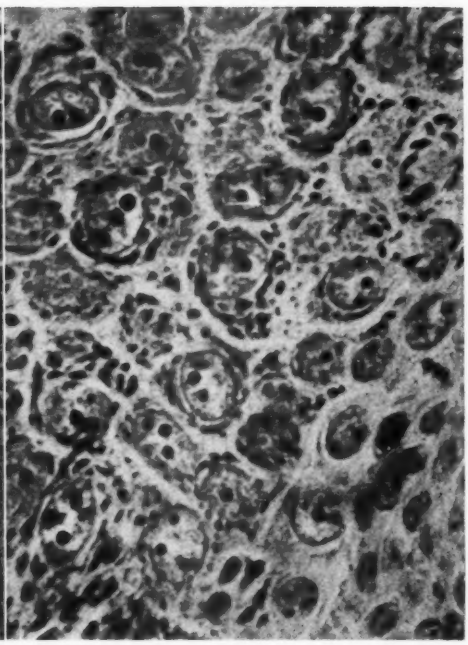

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Bunting, Strauss, and Banfield Skin Papillomas Yielding Virus-Like Particles

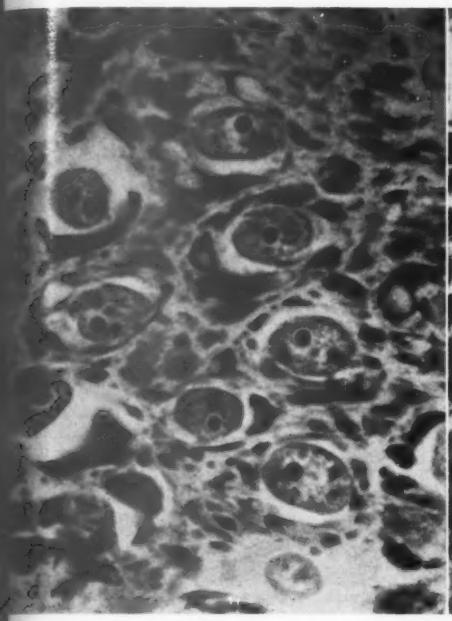
PLATE 153

FIG. 4. The clear halo around each intranuclear inclusion body is evident. In the cells of the upper stratum Malpighii fusion of the multiple cytoplasmic masses occurs as well as increase in size of the vacuoles within them. Hematoxylin and eosin stain. × 800.

FIG. 5. Large block-like cytoplasmic masses in the upper layers of the stratum Malpighii and lower stratum corneum. Mitotic figures have been observed only in unaffected cells. Hematoxylin and eosin stain. × 800.

FIG. 6. Normal cell in midst of affected ones. The base of the wart is at the lower right. The intranuclear inclusion bodies as well as the cytoplasmic masses increase in size as the cells mature. × 800.

FIG. 7. In some instances there are seen single or small groups of affected cells isolated within areas of unaffected ones. Hematoxylin and eosin stain. × 800.



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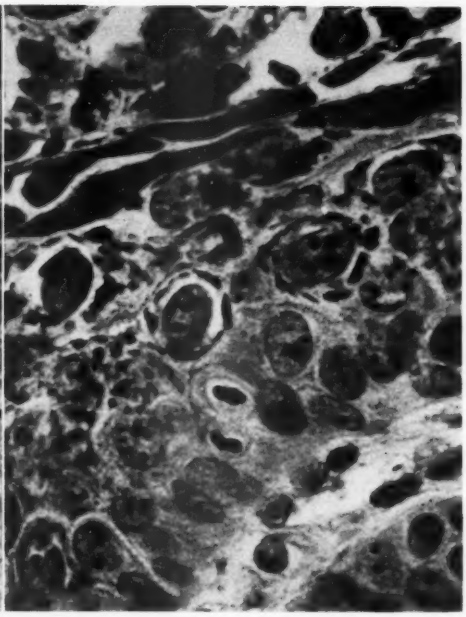

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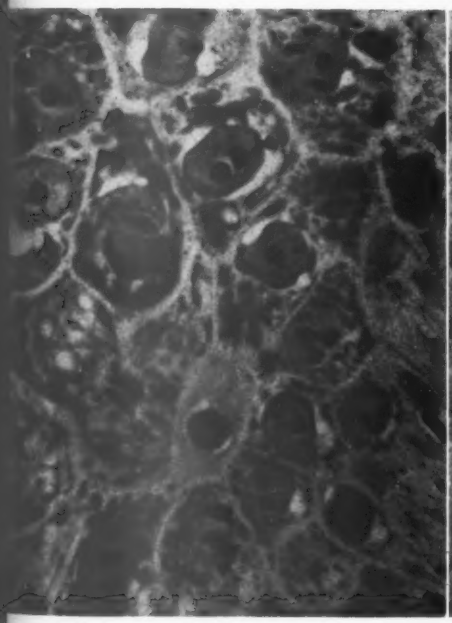

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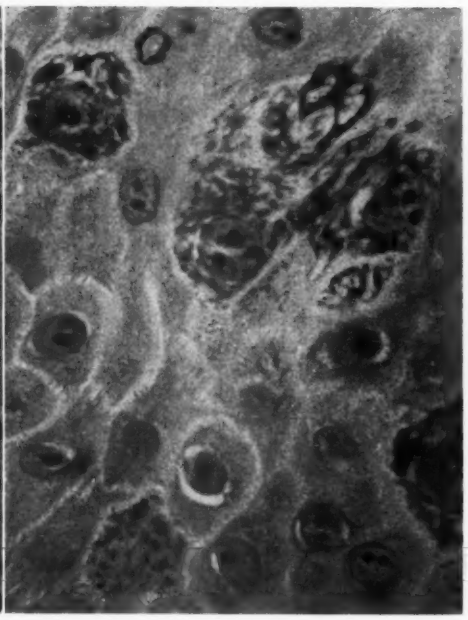

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PLATE 154

FIG. 8. Intercellular bridges present between the unaffected cells are usually absent between two affected cells (at bottom). Aniline blue stain. × 800.

FIG. 9. Feulgen preparation of the cells subsequently stained with hematoxylin and eosin shown in Figure 10. The intranuclear inclusion body, cell to right of center, is Feulgen negative as are the cytoplasmic masses. Nucleoli can usually be identified by rim of Feulgen positive substance. × 800.

FIG. 10. Hematoxylin and eosin preparation showing same cells as in Figure 9. The intranuclear inclusion body and cytoplasmic masses are evident. × 800.

FIG. 11. Photomicrograph of cell taken in light of 2650 Å wave length. Of note is the relative contrast between nuclear membrane and chromatin granules and the intranuclear inclusion body. × 600.

FIG. 12. Photomicrograph of same cell taken in light of 2800 Å wave length. The density of the nuclear membrane and granules relative to that of the intranuclear inclusion body is less than at 2650 Å. × 600.

FIG. 13. Structures resembling "myelin figures" develop from the vacuoles in the cytoplasmic masses after the tissue is exposed to dilute alcohol, suggesting that the masses may contain lipid. Hematoxylin and eosin stain. × 350.

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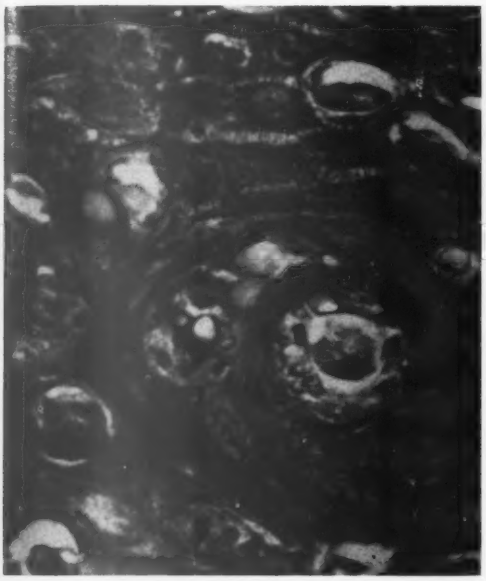

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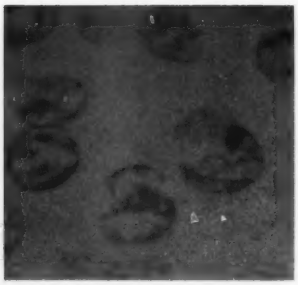

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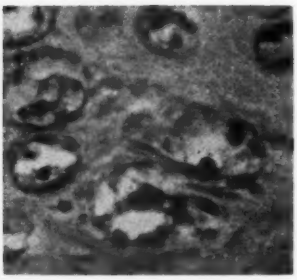

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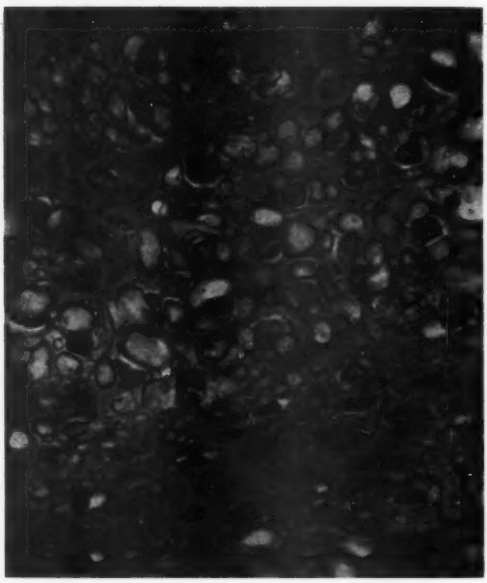

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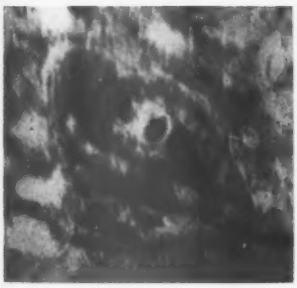

2

BIOPSY OF THE LIVER IN INFECTIOUS MONONUCLEOSIS *

RICHARD C. WADSWORTH, M.D., and PHILIP G. KEIL, Major, USAF(MC)
(From the Department of Pathology, Eastern Maine General Hospital, and the Medical Service, USAF Hospital, Dow Air Force Base, Bangor, Me.)

Because of the relatively benign character of infectious mononucleosis there has been little post-mortem material from which to study the anatomical effects of this disease. There are 24 reported necropsies[1-13] on patients who died either during or shortly after an attack of infectious mononucleosis or glandular fever. Five of these necropsies[1-3] were performed prior to the introduction of the Paul-Bunnell test, so that the diagnoses may be regarded with some suspicion. Most of the necropsy reports agree that there are widespread effects of the disease and the liver usually has shown rather marked alterations. There has been some variation in the reported histologic details of the hepatic lesion. In only one of these fatal cases has death been attributed to hepatic dysfunction.[11]

Numerous articles have emphasized the frequency with which impairment of liver function can be demonstrated in non-fatal cases of infectious mononucleosis with or without jaundice. Most of these reports[14-26] have been based on the abnormal results obtained with various liver function tests. It is rather surprising, in view of such evidence, that there are few available reports of biopsy of the liver from non-fatal cases of this disease. Only seven articles[9,27-32] could be found recording 18 such biopsies from 16 patients with infectious mononucleosis.

The first reported biopsy of the liver in infectious mononucleosis was that described by Kilham and Steigman[27] in 1942. A biopsy specimen was obtained by liver punch from one of the 4 jaundiced patients in their series of 20 cases of infectious mononucleosis. This patient was deeply jaundiced. No mention was made of the time which had elapsed between the onset of symptoms and biopsy. A loss of liver cells, a well developed histiocytic reaction, and some early proliferation of the bile ducts were noted in the portal zones. Increased Kupffer cells and cells resembling monocytes were observed in other parts of the lobules and in the sinusoids. The glycogen content of the hepatic cells was said to be well preserved and the general appearance suggested to the authors that an earlier phase of necrosis had preceded the histiocytic reaction. No peripheral fibrosis was seen, but the re-

* Presented at the Forty-ninth Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 10, 1952.

Received for publication, May 2, 1952.

ticular pattern in the affected portal zones was said to be disturbed. Retention of bile was not mentioned in the report.

The following year van Beek and Haex[28] reported upon 2 specimens obtained by liver puncture from a patient with infectious mononucleosis. The first specimen was taken 14 days after the onset of the illness, and the second 3½ weeks after the onset, when the patient was improved. In the earlier material the liver cells were interspersed with numerous monocytoid cells and some polymorphonuclear leukocytes. Numerous mitotic figures were described in the monocytoid cells. Mitotic figures were seen also in the liver cells. In the later specimen the liver tissue was said to have been practically normal, although the triangles of Kiernan were still rich in lymphocytes. Neither jaundice nor bile retention was mentioned in this report.

Liver biopsies of 4 jaundiced patients with infectious mononucleosis were reported by Bang and Wanscher[29] in 1945. By aspiration, material was obtained from the 4 patients on the 15th, 16th, 6th, and 17th days of illness, respectively. Small, thin, biliary thrombi were observed in the bile capillaries of the first and fourth patients. None were seen in the second and third patients. A periportal infiltration of lymphocytes and a proliferation of Kupffer cells were noted in all 4 cases. In none of these patients was the portal connective tissue considered to be increased. All of the livers showed mild degenerative changes in the hepatic cells. These authors concluded that the jaundice was due not to obstruction, but rather to primary damage to the liver, presumably caused by the specific agent of the disease.

By surgical excision, a specimen of the liver for biopsy was obtained 15 days after the onset of infectious mononucleosis in the course of an operation for a ruptured spleen in the patient reported by Davis, MacFee, Wright, and Allyn.[30] The sections of liver revealed moderate cloudy swelling of the liver cords. Most of the liver cells were swollen, containing fine granules in varying quantity, and a few mitotic figures were present. Several patchy accumulations of pigment were seen in the bile canaliculi. The sinusoids were slightly dilated and contained increased numbers of young lymphocytes, with occasional monocytes and polymorphonuclear cells. The Kupffer cells contained granular pigment in small quantities. No microscopic change was observed in the capsule. This patient apparently was not clinically jaundiced.

The specimen for liver biopsy mentioned in the comprehensive report on the pathology of infectious mononucleosis by Custer and Smith[9] is described only in conjunction with the livers from 7 necropsies. The changes were similar to those seen in the necropsied cases in which death occurred from 17 to 35 days after the onset of the disease.

Lymphocytic infiltration was most pronounced in the periportal connective tissue. "Quantitatively, this reaction varied from hardly more than the usual lymphocytic collar . . . . to a degree approaching that of lymphatic leukemia . . . ., with lymphoid cell aggregates extending into the adjacent lobular parenchyma." The capsule in most instances showed varying degrees of lymphocytic infiltration while its connective tissue appeared to be losing substance in proportion to the increase of lymphocytes. These authors saw no necrosis of the liver parenchyma except in one case in which thrombosis of the portal vein followed splenectomy. They saw no evidence of biliary obstruction. The incidence of jaundice in these 8 cases is not mentioned.

Results of biopsy of the livers of 5 young adults taken at the height of typical attacks of infectious mononucleosis without clinical evidence of hepatic disturbance were reported by Bertrand[31] in 1949. In one patient, whose recovery was delayed, specimens were obtained at 1 and 5 months after the onset of the disease. In each case there was a marked infiltration of the periportal connective tissue with mononuclear cells. There were increased mitotic figures in the liver cells. The blood vessels, bile ducts, and liver cells were essentially unaltered. There was marked hyperplasia of the Kupffer cells. Islets of necrosis were attributed to the displacement of trabeculae by masses of lymphocytes. Mitotic figures were found in the Kupffer cells and in the periportal mononuclear cells. Similar findings in specimens obtained for biopsy from 2 additional patients were recorded in a footnote.

The only report suggesting that cirrhosis of the liver might be a complication of infectious mononucleosis is that of Leibowitz and Brody.[32] They described a 24-year-old white male who was admitted to the hospital with jaundice of 7 weeks' duration. Fifty-nine days after the onset of jaundice, the heterophil antibody titer reached a peak of 1:800. Thirty-nine months after the onset of jaundice, material obtained by aspiration for biopsy of the liver revealed microscopic evidence of cirrhosis.

Material and Methods

During the 8-month period between June 15, 1951, and February 15, 1952, the diagnosis of infectious mononucleosis was made on 26 patients. Thirteen specimens obtained from 10 of these patients have provided the opportunity to compare the histologic changes in the liver with the clinical course of the patient, the heterophil antibody titers, the smears of the peripheral blood, and certain liver function tests. The specimens were all obtained from young adult males between the ages of 18 and 31 years. Liver tissue was obtained with

biopsy forceps during peritoneoscopy. With this method the liver could be seen and described and the site could be visualized before and after the tissue was removed. In each case the sample was taken from the right lobe of the liver.

The tissue removed was placed immediately in 10 per cent neutral formalin solution. Paraffin sections were stained with hematoxylin and eosin and with Gomori's silver stain for reticulum.[33] In some cases a portion was fixed in Zenker's fluid and stained with phloxine and methylene blue.

The heterophil antibody titer was determined according to the presumptive test of Davidsohn.[34] An agglutination in a final serum dilution of 1:224 or higher was considered significant. Absorption tests with guinea-pig kidneys and beef erythrocytes were not done.

Cephalin-cholesterol flocculation tests were done by the technic of Hanger.[35] Readings were recorded at the end of 24 and 48 hours. A reaction of 2 plus or more in 24 hours or of 3 plus or more in 48 hours was considered abnormal.

The thymol turbidity test was performed by Benotti's modification[36] of the Maclagan method with the reagents buffered at pH 7.8, measuring the turbidity in a colorimeter against standard barium chloride solutions. A value of more than 2 units was considered abnormal.

Total and direct bilirubin determinations were done by the method of Malloy and Evelyn.[37] Elevation of the total bilirubin above 1.0 mg. per 100 cc. was considered abnormal.

Total and free cholesterol determinations were done by the method of Schoenheimer and Sperry.[38] A value of less than 70 per cent for cholesterol esters was considered abnormal.

Alkaline phosphatase determinations were done by the method of Bodansky.[39] Values greater than 4 Bodansky units were considered abnormal.

Bromsulfalein retention was measured by the injection of 5 mg. of the dye per kg. of body weight. A retention of more than 5 per cent at the end of 45 minutes after injection was considered abnormal.

Results

Group I

Group I consisted of 2 patients from whom specimens were obtained for biopsy within 5 days of the onset of symptoms.

Case 1, a 24-year-old white male, typifies this group. The specimen was taken 2 days after the onset of severe pharyngitis with fever of 102.0° F. On the day of the biopsy his leukocyte count was 18,500 per cmm. with 74 per cent neutrophils and 26 per cent lymphocytes.

Three days later his heterophil antibody titer was 1:224. Thirteen days after the biopsy his peripheral blood contained 56 per cent lymphocytes, some of which were atypical. The spleen was palpable. There was no jaundice.

At the time of biopsy the liver was moderately enlarged, extending 4 cm. below the costal margin all along the hypogastrium. The gallbladder appeared normal. The liver edge was somewhat blunted, and its color was distinctly lighter than normal. Histologically, the capsule appeared intact except for a few scattered epithelioid cells. Only occasional lymphocytes were found in the periportal zones. The liver cords were well preserved. Occasional liver cells were somewhat shrunken, with pyknotic nuclei. There was some variation in the size and staining of the nuclei. Occasional liver cells were binucleate, with prominent nucleoli. Small, clear vacuoles were found in many of the liver cells. The Kupffer cells were not numerically increased, but some of them appeared swollen. Some of the liver cells had indistinct cell membranes and occasional neutrophils were found within the parenchyma as well as in the sinusoids. No mitotic figures were seen.

Case 2 was a 20-year-old white male from whom a specimen for biopsy was obtained 5 days after the onset of acute pharyngitis. Three days prior to the biopsy he had a leukocyte count of 18,700 per cmm. with 86 per cent neutrophils and 14 per cent lymphocytes. No atypical lymphocytes were demonstrated in the peripheral blood of this patient. On the day of biopsy his heterophil antibody titer was 1:112. Seven days later it was 1:224. There was no jaundice.

At the time of biopsy the liver was moderately enlarged, its edge was rounded, and its color was slightly lighter than normal. Histologically, the liver was similar to that of case 1. More of the liver cells were multinucleated. Some had three or four nuclei. The Kupffer cells were increased in both size and number. The periportal zones were somewhat accentuated by an infiltration of epithelioid cells, some lymphocytes, and occasional neutrophils. Scattered small foci of Kupffer cells appeared to be replacing liver cells in some areas.

Group II

Group II consisted of 4 patients from whom specimens for biopsy were obtained between 10 and 15 days after the onset of symptoms. Case 3 is representative of the group.

Case 3 was a 20-year-old white male from whom tissue was obtained 10 days after the onset of acute pharyngitis with lymphadenopathy and fever. He had no symptoms referable to his liver. Six days prior to the biopsy his leukocyte count was 12,250 per cmm. with 26

per cent neutrophils, 68 per cent lymphocytes, and 6 per cent monocytes. Many of the lymphocytes were designated "mononucleosis cells." There was no jaundice.

At the time of biopsy the liver appeared to be of normal size and its edge sharp. The color and texture appeared normal. The liver extended just to the level of the right costal margin. The capsule was smooth and glistening. The color was uniform and of a mahogany hue. Histologically, the liver showed a striking difference from those of group I. The capsule appeared thin. In some parts of the capsule there was a slight infiltration of lymphocytes and epithelioid cells. The portal zones were markedly accentuated by a dense infiltration of lymphocytes and epithelioid cells (Fig. 1) and a few scattered neutrophils and eosinophils. There was a moderate increase in bile ducts and a slight fibroblastic proliferation. The sinusoids appeared small but were conspicuous because of the numerous and swollen Kupffer cells which outlined them. Some of the Kupffer cells seemed to be free in the lumina of the sinusoids. Most of the parenchymal cells contained a faintly eosinophilic granular cytoplasm. Many of these cells had two or more nuclei. An occasional nucleus was elongated, with a slight constriction near the mid-point. Occasional mitotic figures were seen in the liver cells. Scattered cells and sometimes small groups of liver cells appeared shrunken, irregular in outline, and deeply eosinophilic, with small, dark-staining, homogeneous nuclei (Fig. 2). Some cells with the more intensely eosinophilic cytoplasm had small, irregular, pyknotic nuclei. A few cells were hyalinized, deeply eosinophilic, and without demonstrable nuclei (Fig. 3). Some of these cells appeared to be extruded from the liver cell columns. In some of the liver lobules many of the liver cells had large, clear nuclei suggesting excessive glycogen content (Fig. 4). An occasional small, irregular group of epithelioid cells appeared to occupy the space from which two or three liver cells had disappeared. Some of the liver cells near the central veins contained small granules of light brown pigment. No bile plugs were formed in the bile canaliculi. No mitotic figures were found in the Kupffer cells. An occasional mitotic figure was seen in the epithelium lining the bile ducts. Numerous lymphocytes were seen in the lumina of the sinusoids.

Case 4 was a 23-year-old white male from whom material for biopsy was obtained 11 days after the onset of acute pharyngitis. Three days before biopsy his leukocyte count was 10,500 per cmm. with 22 per cent neutrophils, 65 per cent lymphocytes, 2 per cent monocytes, and 11 per cent staff cells. Atypical lymphocytes were seen in the smear. On the day of biopsy the heterophil antibody titer was 1:224 and the

cephalin-cholesterol flocculation test was negative at 24 and 48 hours. Serial laboratory examinations were not obtained on this patient. There was no jaundice.

At the time of biopsy the liver appeared grossly normal. Histologically, it (Fig. 5) was quite similar to that seen in case 3. The periportal lymphocytic infiltration and Kupffer cell activity were not quite as marked but the acidophilic degeneration of individual hepatic cells was more pronounced.

Case 5 was an 18-year-old white male upon whom biopsy was performed 13 days after the onset of acute pharyngitis associated with fever, lymphadenopathy, and splenomegaly. One week prior to the biopsy his leukocyte count was 11,100 per cmm. with 38 per cent neutrophils, 2 per cent eosinophils, 55 per cent lymphocytes, and 5 per cent monocytes. Atypical lymphocytes were seen in the smear. At the time of the biopsy the heterophil antibody titer was 1:224. The cephalin-cholesterol flocculation test was 1 plus at 24 hours and 2 plus at 48 hours. Six days later the cephalin-cholesterol flocculation test was negative at 24 and 48 hours. The spleen was palpably enlarged. There was no jaundice.

At the time of biopsy the liver was slightly enlarged. The color was uniformly light pink. There was some blunting of the mesial edge of the right lobe. Histologically, the liver was similar to that of case 3. The periportal infiltration was not quite as marked and the Kupffer cell activity was not nearly as marked. There was a moderate acidophilic degeneration of individual hepatic cells, but no evidence of mitotic activity in the liver cells.

Case 6 was a 21-year-old white male from whom tissue for biopsy was obtained 14 days after the onset of chills, weakness, and anorexia. When first seen he had a single extensive chain of enlarged cervical lymph nodes. Six days prior to the biopsy his leukocyte count was 11,000 per cmm. with 48 per cent neutrophils, 1 per cent staff cells, 48 per cent lymphocytes, and 3 per cent monocytes. Atypical lymphocytes were seen in the smear. Four days prior to biopsy the heterophil antibody titer was 1:7168. On the day after biopsy the cephalin-cholesterol flocculation test was 1 plus in 24 hours and 3 plus in 48 hours. The same sample of blood showed an elevated thymol turbidity of 4.2 units. Repeat tests performed 8 and 15 days respectively after the biopsy showed no abnormalities. There was no jaundice.

At the time of biopsy the liver was slightly enlarged and its edge blunted. The color was a brilliant deep red. Histologically, the liver was similar to that of case 3. The periportal infiltration and Kupffer cell activity were not quite as marked but the acidophilic degeneration

of individual liver cells and the mitotic activity of the liver cells were fairly marked. In scattered areas there was excessive glycogen deposition in the nuclei of the liver cells. There was moderate increase in the bile ducts.

Group III

Group III included only case 8, a 21-year-old white male whose course was more protracted than that of the previous cases. His biopsy specimen was obtained 28 days after the onset of severe pharyngitis and marked lymphadenopathy. Three days after the onset his leukocyte count was 7,500 per cmm. with 22 per cent neutrophils, 28 per cent staff cells, 33 per cent lymphocytes, 9 per cent monocytes, 6 per cent eosinophils, and 2 per cent basophils. No atypical lymphocytes were seen at this time. Fourteen days after onset his leukocyte count was 18,200 per cmm., with 1 per cent neutrophils, 13 per cent staff cells, 79 per cent lymphocytes, and 7 per cent monocytes. Most of the lymphocytes were atypical.

At the time of biopsy his liver was only slightly enlarged but the liver edge was definitely blunted. The color was a brilliant deep red. Histologically, the liver was similar to that of case 3. The periportal infiltration and Kupffer cell activity were not quite as pronounced. The acidophilic degeneration of individual liver cells was quite marked.

Group IV

Group IV includes 3 patients from whom more than one specimen of liver was obtained. Case 7 was a 20-year-old white male with a history of pharyngitis, fever, cervical lymphadenopathy, enlarged spleen, and tender liver. Three days after the onset his leukocyte count was 14,700 per cmm. with 15 per cent neutrophils and 85 per cent lymphocytes, most of which were atypical. The first liver biopsy was performed 19 days after the onset of his illness. At that time the liver was somewhat enlarged. Its edge was considerably blunted and its color a brilliant red. Histologically, the liver was quite similar to case 3 but the infiltration extended into the liver lobules (Fig. 6). The mitotic activity of the liver cells was extremely marked (Fig. 7). More liver cells were in mitosis than in any other specimen of the entire series. There was a moderate multiplication of the bile ducts (Fig. 8). Occasional mitotic figures could be seen in epithelial cells lining the bile ducts. There was moderate acidophilic degeneration of individual liver cells. The second liver specimen from case 7 was obtained 59 days after the onset of illness. The liver appeared slightly larger than normal and its edge was slightly blunted. The color was slightly lighter than normal. The site of the previous removal was

noted and the second specimen was obtained adjacent to the site of the first. There was a striking difference histologically between the two. Periportal infiltration and Kupffer cell activity were much less marked in the second (Fig. 9). Only an occasional mitotic figure could now be found in the liver cells. There were only a few liver cells showing degenerative changes. A significantly elevated heterophil antibody titer persisted for at least 42 days.

Case 9 was a 31-year-old white male with a history of pharyngitis, fever, and cervical lymphadenopathy. Three days after onset the leukocyte count was 14,150 per cmm. with 18 per cent neutrophils, 77 per cent lymphocytes, and 5 per cent monocytes. Many of the lymphocytes were atypical. Eight days after the onset the heterophil antibody titer was 1:3584 and 20 days later was 1:7168. In the meantime the cephalin-cholesterol flocculation test was 4 plus in 24 hours and the thymol turbidity was 9.4 units. The total cholesterol was 225 mg. per 100 cc. with 74 per cent esters. The patient was jaundiced and the total bilirubin was 2.5 mg. per 100 cc. The first liver biopsy was done 57 days after the onset of illness when the cephalin-cholesterol flocculation test and thymol turbidity were still elevated, although the bilirubin had reached normal levels. At this time the liver was slightly larger than normal. It had lost some of its brownish tint and was smooth without evidence of scarring or fatty infiltration. Histologically, there was only slight evidence of active inflammation. There was very little periportal infiltration and very little Kupffer cell activity (Fig. 10). Only occasional liver cells showed acidophilic degeneration. A few liver cells had multiple nuclei. No mitotic figures were seen. There was no evidence of periportal scarring. The second specimen for biopsy was obtained 121 days after the onset of illness. The patient was still presenting symptoms of his disease. He showed no retention of bromsulfalein at the end of 45 minutes. The liver appeared nearly the same as before, both grossly and histologically (Fig. 11). The Kupffer cells seemed slightly more active and there were more liver cells with multiple nuclei and prominent nucleoli. There now appeared to be very slight periportal fibroblastic proliferation and some increase in bile ducts. There was slight retention of bile in some of the canaliculi.

Case 10 was a 31-year-old white male with a protracted history starting with a 2-day period during which his friends noticed that he was jaundiced. He then felt perfectly well for approximately 2 months when he developed nausea and vomiting and noticed that his sclerae were yellow and his urine dark. Five days later the total bilirubin was 5.4 mg. and the direct reacting bilirubin 3.0 mg. per 100 cc. The urinary urobilinogen at this time was positive in a dilution of 1:640.

The alkaline phosphatase was 6.2 Bodansky units but the cephalin-cholesterol flocculation was negative at 24 and 48 hours, and the bromsulfalein retention was only 2.5 per cent in 45 minutes. Two weeks before the second biopsy the total serum cholesterol was 149 mg. per 100 cc. with 75 per cent esters. The first liver biopsy was done 120 days after the first episode of jaundice. For 6 days prior to biopsy he had received 40 mg. of ACTH every 6 hours. At that time the cephalin-cholesterol flocculation and thymol turbidity values were still elevated. The bilirubin was 1.5 mg. per 100 cc. The liver appeared normal by peritoneoscopy. Histologically, the periportal infiltration and Kupffer cell activity were minimal. A few scattered liver cells showed degenerative changes. There were a moderate number of binucleate liver cells. No mitotic figures were seen. Retained bile could be seen in some of the bile canaliculi (Fig. 12). There was no evidence of periportal scarring. The second biopsy was done 225 days after the first episode of jaundice. The total bilirubin was then 1.2 mg. per 100 cc. At that time the liver appeared normal at peritoneoscopy. Histologically, the liver now showed moderate Kupffer cell activity. Clear vacuoles were demonstrable in occasional liver cells. Most of the liver cells had a fairly uniform appearance, but there was a moderate variation in the size and staining ability of the nuclei (Fig. 13). A moderate number of binucleate liver cells were seen. There was an occasional mitotic figure. There were scattered small focal areas of necrosis of liver cells. Small collections of retained bile could be seen in some of the small bile canaliculi. There was very slight periportal scarring. The reticular pattern of the liver lobules was, for the most part, well maintained (Fig. 14).

Discussion

The study of biopsy specimens from the livers of 10 patients with infectious mononucleosis offers additional evidence of liver damage in this disease. In each patient upon whom biopsy was done, the liver showed morphologic evidence of liver damage. This varied considerably both in the type and intensity of the reaction, as shown in Table I. In those patients from whom specimens were obtained within 5 days of the onset of symptoms (group I), the pathologic changes in the liver were minimal. In case 1 the tissue appears to have been taken almost at the peak of the heterophil antibody titer, but 2 weeks prior to the development of a positive cephalin-cholesterol flocculation test. A repeat biopsy 2 weeks later might have shown more marked pathologic changes. Case 2 is an example of a mild case of infectious mononucleosis with minimal changes in the liver.

In those specimens obtained 10 to 30 days after the onset of symptoms (cases 3 to 8) there was evidence of marked periportal infiltration, marked Kupffer cell activity, and considerable acidophilic degeneration of individual liver cells. It is to be noted that the maximum mitotic activity of liver cells was seen in the specimen obtained 19 days after the onset of symptoms. In this group we find there was

TABLE I

Summary of 13 Biopsies of the Liver in 10 Patients

Case no.	Elapsed days	Heterophil antibody	Cephalin-cholesterol flocculation		Thymol turbidity	Total bilirubin	Periportal infiltration	Kupffer cell activity	Hepatic cell destruction	Mitosis of hepatic cells
			240°	480°						
1	2	224	2	3	1.9	0.8	1	1	1	0
2	5	224	0	1	2.2	1.0	2	2	1	0
3	10	224	1	2	1.7	0.6	4	4	2	1
4	11	224	0	0			3	3	3	1
5	13	224	0	0			3	2	2	0
6	14	7168	1	3	4.2		3	3	2	2
7A	19	1792	3	4	3.1	0.7	4	3	2	3
8	28	1792	3	4	4.6	1.4	3	3	2	1
9A	57	7168	4	4	9.4	2.5	2	2	2	0
7B	59	1792	3	4	3.1	0.7	2	2	2	1
10A	120	1792	2	3	3.1	5.4	1	1	1	0
9B	121	7168	4	4	9.4	2.5	2	2	1	0
10B	225	1792	2	3	3.1	5.4	1	3	2	1

For each case are shown the days elapsed since onset of symptoms, the highest recorded heterophil antibody titer, the highest cephalin-cholesterol flocculation reading, the highest thymol turbidity, the most elevated total serum bilirubin, the intensity of the periportal mononuclear infiltration (graded 1 to 4), the intensity of Kupffer cell activity (graded 1 to 4), the amount of hepatic cell destruction (graded 1 to 4), and the degree of mitotic activity in the liver cells (graded 1 to 4).

an apparent lack of correlation between the structural changes in the liver and liver function tests. In case 3 the histologic changes on the tenth day were striking but the cephalin-cholesterol flocculation tests, the thymol turbidity tests, and bilirubin determinations were all within normal limits on the 7th, 15th, 19th, and 28th days after the onset of symptoms. In cases 4 and 5, in which there was histologic evidence of moderate liver damage, the liver function tests showed no abnormalities although they were done within 3 days of the date of biopsy. In case 6 the cephalin-cholesterol flocculation and thymol turbidity tests were slightly elevated the day after biopsy, but the former became negative within a week after biopsy. In case 8 we had the best correlation between the high heterophil titer, elevated cephalin-cholesterol flocculation and elevated thymol turbidity, and the histologic

appearance of an active hepatitis. It is of interest that the thymol turbidity remained elevated for 1 month after the cephalin-cholesterol flocculation test became negative. When thymol turbidity reached its peak, the total serum bilirubin was 1.4 mg. per 100 cc. The patient was never jaundiced clinically.

In case 7, with biopsies on the 19th and 59th days after the onset of symptoms, there was an initial correlation between the hepatic histologic picture and the liver function tests. When the first biopsy was taken the heterophil antibody titer was 1:1792 and the cephalin-cholesterol flocculation test was abnormal. A few days later the thymol turbidity test became abnormal. However, there were histologically demonstrable liver lesions at the time of the second biopsy when the liver function tests were within normal limits.

In case 9 the histologic changes, although relatively slight, persisted for some time after the cephalin-cholesterol flocculation was negative. In spite of the protracted course of this patient, he had no bromsulfalein retention 3 months after the onset of his illness.

In case 10 the first biopsy, 120 days after the onset of symptoms, showed only minimal histologic change in spite of elevated cephalin-cholesterol flocculation and thymol turbidity tests. The total serum bilirubin at this time was 1.5 mg. per 100 cc. In spite of the prolonged course, the liver showed only slight residual structural damage. The patient continued to show mitotic activity in the liver cells at the time of the second biopsy.

The study of these liver specimens taken at intervals from 2 to 225 days after the onset of infectious mononucleosis suggests an explanation for the discrepancy in previously reported histologic findings in this disease. As might be expected, the histologic features appear to depend upon the time interval which has elapsed since the onset of the disease, upon the severity of the infection and, to some extent, upon the presence or absence of jaundice. Only minimal changes in the liver were found in the first 5 days of the disease. They consisted of slight lymphocytic infiltration in the periportal zones, degenerative changes in the liver cells, evidence of regeneration of liver cells, and activity of the Kupffer cells. After 10 days these processes all appeared to be more active. The peak of degenerative and regenerative activity was reached between the 10th and 30th days. During this period periportal infiltration and Kupffer cell activity were most pronounced. At the end of 60 days the periportal infiltration and Kupffer cell activity had subsided considerably but were still present in the protracted cases. Some evidence of isolated degeneration and regeneration of liver cells was still to be seen as long as 225 days after onset. The

histologic changes in the liver are similar to those described in non-fatal cases of epidemic hepatitis.[40] Endophlebitis or inclusion bodies were not seen.

Summary

Liver specimens were obtained by forceps, at peritoneoscopy, in 10 patients with infectious mononucleosis. Biopsies were done from 2 to 225 days after the onset of symptoms. The gross and histologic findings are similar to those described in the livers of patients with non-fatal epidemic hepatitis. Serial liver function studies were performed on most of these patients. An attempt has been made to correlate the histologic findings with the clinical course and the laboratory data. The degree of anatomical change does not parallel the degree of abnormality of the liver function tests.

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[*Illustrations follow*]

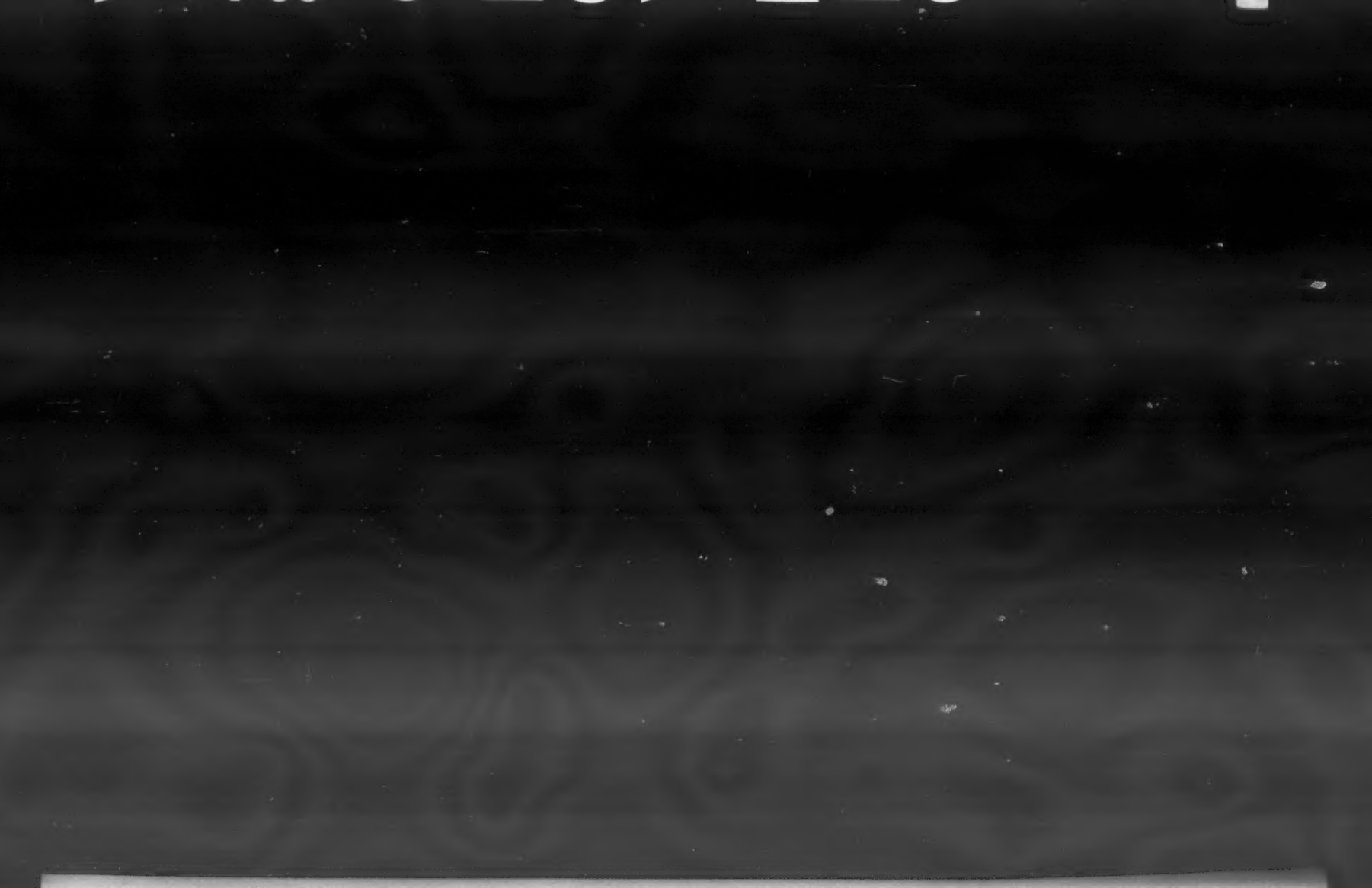
DESCRIPTION OF PLATES

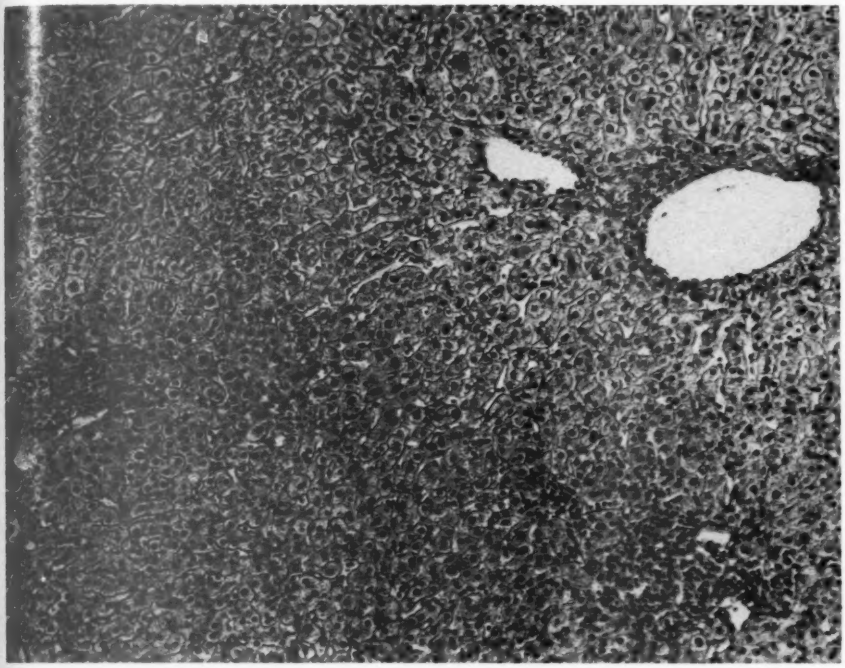
PLATE 155

FIG. 1. Liver, case 3 (K. L. M.), 10 days after onset. Periportal mononuclear infiltration with fair preservation of lobular architecture. Hematoxylin and eosin stain. × 115.

FIG. 2. Liver, case 4 (C. L. W.), 11 days after onset. Early stages of acidophilic degeneration of individual hepatic cells. Some cells show hydropic degeneration. Hematoxylin and eosin stain. × 950.

FIG. 3. Liver, case 4 (C. L. W.), 11 days after onset. Late stage (hyalinization) of acidophilic degeneration of individual hepatic cells. Mononuclear cells in sinusoids. Hematoxylin and eosin stain. × 950.





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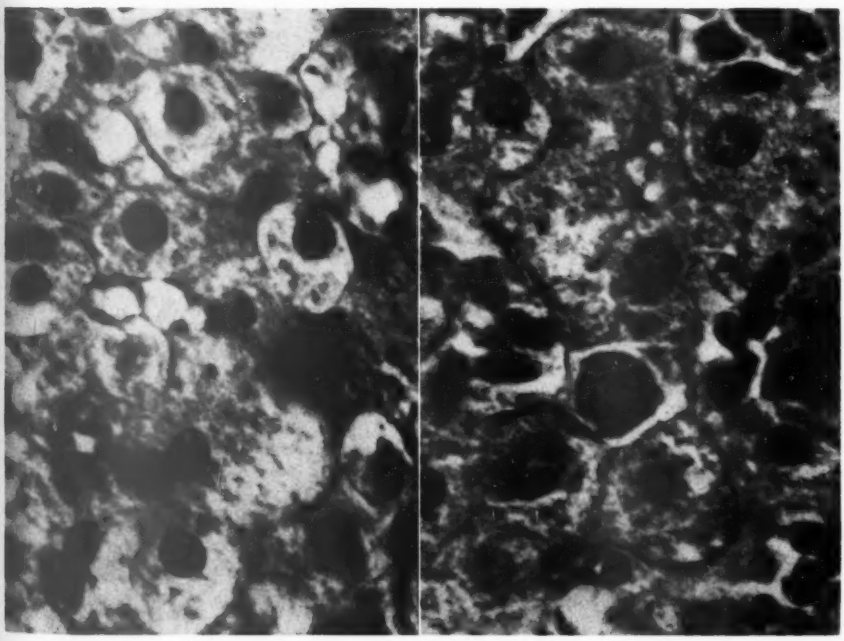

3

Wadsworth and Keil Liver in Infectious Mononucleosis

PLATE 156

FIG. 4. Liver, case 3 (K. L. M.), 10 days after onset. Numerous hepatic cell nuclei with excessive glycogen content. Binucleate liver cells. Kupffer cells increased in size and number. Hematoxylin and eosin stain. × 940.

FIG. 5. Liver, case 4 (C. L. W.), 11 days after onset. Periportal mononuclear infiltration. Moderate variation in size, shape, and staining reaction of hepatic cells and nuclei. Hematoxylin and eosin stain. × 115.

FIG. 6. Liver, case 7 (L. G. H.), 19 days after onset. Mononuclear infiltration not only in periportal areas but also in scattered foci throughout the liver lobule. Hematoxylin and eosin stain. × 115.

FIG. 7. Liver, case 7 (L. G. H.), 19 days after onset. Binucleate hepatic cells and three mitotic figures in hepatic cells. Hematoxylin and eosin stain. × 950.

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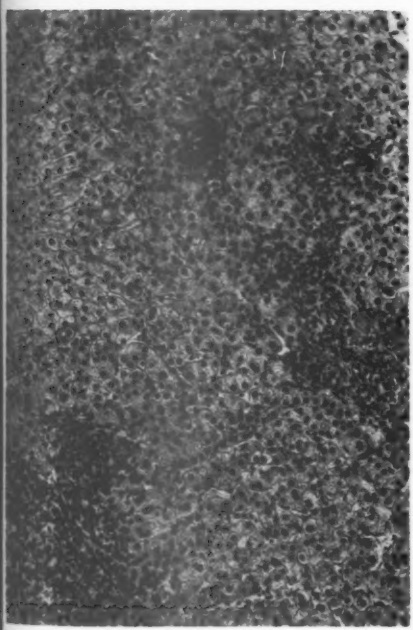

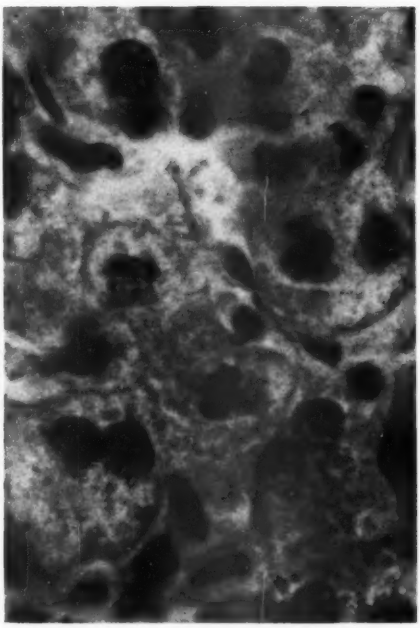

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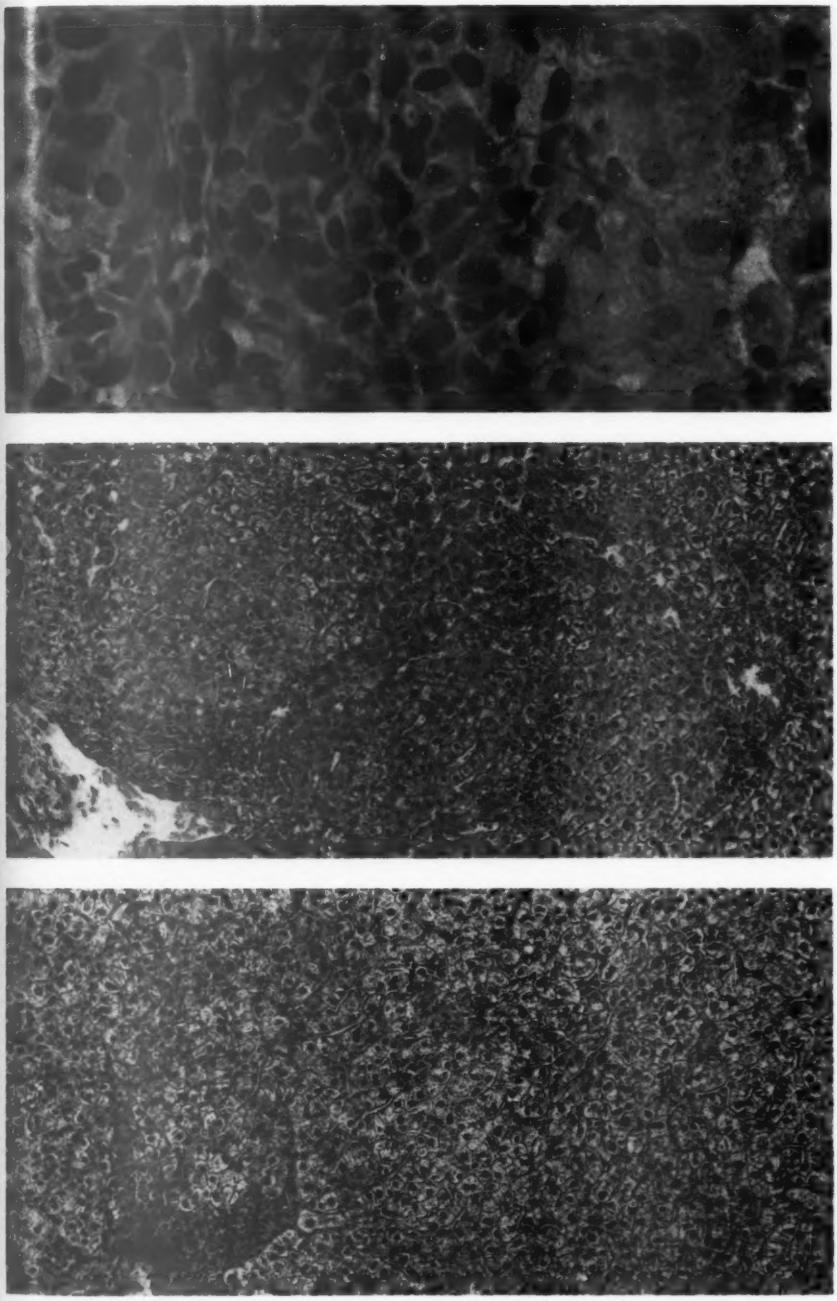
Wadsworth and Keil Liver in Infectious Mononucleosis

PLATE 157

FIG. 8. Liver, case 7 (L. G. H.), 19 days after onset. Marked periportal mononuclear infiltration. Reduplication of bile ducts. Hematoxylin and eosin stain. × 900.

FIG. 9. Liver, case 7 (L. G. H.), 59 days after onset. Marked reduction in periportal mononuclear infiltration. Reduction in Kupffer cell activity. Hematoxylin and eosin stain. × 115.

FIG. 10. Liver, case 9 (E. R. D.), 57 days after onset. Slight periportal mononuclear infiltration. A few binucleate liver cells. Hematoxylin and eosin stain. × 115.



Wadsworth and Keil Liver in Infectious Mononucleosis

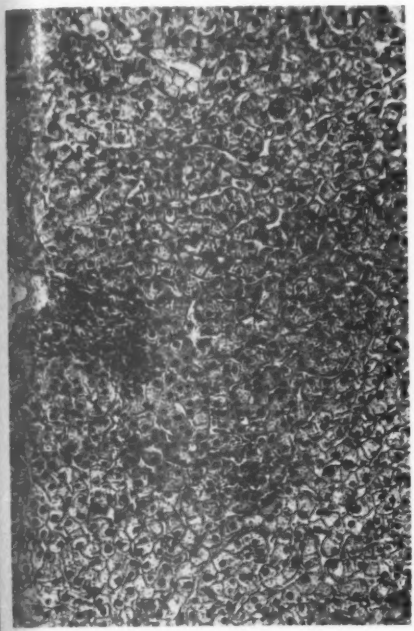
PLATE 158

FIG. 11. Liver, case 9 (E. R. D.), 121 days after onset. Slight periportal mononuclear infiltration; for comparison with Figure 10. Hematoxylin and eosin stain. × 115.

FIG. 12. Liver, case 10 (W. G. E.), 120 days after onset. Bile retention in liver cells and small bile canaliculi about central vein. Binucleate liver cells. Hematoxylin and eosin stain. × 420.

FIG. 13. Liver, case 10 (W. G. E.), 225 days after onset. Practically no periportal mononuclear infiltration. Moderate number of binucleate liver cells. Moderate variation in size and staining of liver cell nuclei. Hematoxylin and eosin stain. × 115.

FIG. 14. Liver, case 10 (W. G. E.), 225 days after onset. Lobular pattern well preserved. Gomori's silver stain for reticulum. × 250.



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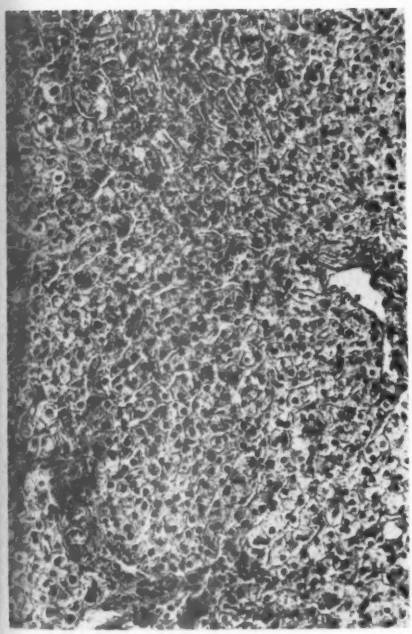

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Wadsworth and Keil

Liver in Infectious Mononucleosis

THE OCCURRENCE AND DISTRIBUTION OF GLYCOGEN IN HEMANGIOMA, DERMATOFIBROSARCOMA PROTUBERANS, HEMANGIOPERICYTOMA, AND KAPOSI'S SARCOMA *

RAYMOND BANGLE, JR., M.D.

(From the Federal Security Agency, U.S. Public Health Service, National Institutes of Health, Bethesda 14, Md.)

The only known extensive report on the topical distribution of glycogen in human tumors is that by Lubarsch[1] in 1906. Using iodine methods or Best's carmine stain, he examined 1544 various tumors, 447 of which contained glycogen microscopically. He found that fibromas, osteomas, gliomas, hemangiomas, and "colloid" carcinomas were free of glycogen. In contrast, glycogen was constantly present in teratomas, "rhabdomyomas," hypernephromas, and chorio-epitheliomas, often abundant in squamous cell carcinomas, and much less frequent in adenocarcinomas. It was his opinion that only tissues normally containing glycogen give rise to glycogen-containing tumors. If this is so, the finding of glycogen in anaplastic malignant tumors might be of value in the histogenetic classification of these tumors.

The recent development of a sensitive method for the demonstration of glycogen in tissue sections has led to a general inquiry into the occurrence of glycogen in human tumors. The periodic acid-leukofuchsin reaction, introduced independently by McManus,[2] Lillie,[3] and Hotchkiss,[4] has proved to be a satisfactory and convenient method for the coloration of glycogen. It is by no means specific for glycogen but may color a number of substances that contain the –CHOH–CHOH–, –CHOH–$CHNH_2$–, or –CHOH–CHNHR– groupings. The split products of glycogen resulting from diastase digestion are not colored by the periodic acid-leukofuchsin reaction. Therefore, by comparing two serial sections, one with preliminary diastase digestion and one without it, it is possible to distinguish between glycogen and other substances subsequently colored by the periodic acid-leukofuchsin method.

This paper deals with the distribution of glycogen in the capillary, cavernous, and arterial hemangiomas, sclerosing hemangioma,[5,6] dermatofibrosarcoma protuberans,[7] hemangiopericytoma,[8] and Kaposi's sarcoma. In addition, normal vascular structures, arrector pili muscle, and fibrous tissue were studied.

MATERIALS AND METHODS

The number of specimens and types of lesions examined are enumerated in Table I. In addition, normal tissue and granulation tissue

* Received for publication, May 5, 1952.

from human skin were studied to determine the occurrence of glycogen in vascular endothelium, vascular smooth muscle, arrector pili muscle, and fibroblasts of normal connective tissue, of scar tissue, and of granulation tissue.

Fixation. All tissues were fixed in 10 per cent neutral formalin within 3 hours after surgical removal. A few specimens were fixed in alcohol-formalin (10 per cent formalin in 95 per cent alcohol). The tissue blocks were embedded in paraffin and sections cut 6 to 7 μ thick.

TABLE I

Glycogen in Hemangiomas, Dermatofibrosarcoma Protuberans, Hemangiopericytoma, and Kaposi's Sarcoma

Lesion and anatomical structure	No. of specimens†	Glycogen			
		3 plus*	2 plus	1 plus	None
Capillary hemangioma					
Endothelium	11	3	3	5	0
Cavernous hemangioma					
Endothelium	4	0	0	3	1
Arterial hemangioma					
Endothelium	2	1	1	0	0
Smooth muscle of arteries		1	1	0	0
Sclerosing hemangioma					
Endothelium, non-lipid-containing histiocytes, and fibroblasts	23	6	6	6	5
Dermatofibrosarcoma protuberans					
Endothelium	4	0	0	3	1
Fibroblasts		3	0	0	1
Hemangiopericytoma					
Endothelium	2	0	1	1	0
Tumor cells (pericytes?)		0	0	0	2
Kaposi's sarcoma					
Endothelium	2	0	1	1	0
Fibroblasts		1	1	0	0

* 3 plus = large amount and diffuse; 2 plus = moderate amount and diffuse; 1 plus = slight amount and diffuse, or focally distributed; none = glycogen not demonstrated.

† The specimens of dermatofibrosarcoma protuberans, hemangiopericytoma, and Kaposi's sarcoma, and a few of the hemangiomas were obtained from the Memorial Center for Cancer and Allied Diseases, New York, N.Y. The remaining specimens of hemangioma were obtained from the National Institutes of Health, Bethesda, Md.

Staining Methods. The periodic acid-leukofuchsin reaction was performed according to the method of Lillie[9] except that potassium metaperiodate was substituted for sodium periodate. The technic of this modification has been presented by Mowry and Bangle.[10] The sections were counterstained with hematoxylin for nuclear detail. Adjacent serial sections were stained by Best's carmine method.[9]

Diastase Digestion Procedure. Prior to staining by periodic acid-leukofuchsin and by Best's carmine, control serial sections were incubated for 1 hour at 37° C. in 0.1 per cent malt diastase (U.S.P. IX) freshly dissolved in saline-phosphate buffer at pH 6.0.[11] The control procedure was performed on all specimens.

With both methods glycogen was identified in the tissue sections as bright red granules that were absent in the control sections after diastase digestion.

Results

The occurrence and distribution of glycogen in hemangiomas, dermatofibrosarcoma protuberans, hemangiopericytoma, and Kaposi's sarcoma are tabulated in Table I. Both the periodic acid-leukofuchsin reaction and Best's carmine method appeared equally sensitive for coloring varying amounts of glycogen. Best's carmine method was superior only in that it stained fewer tissue components other than glycogen. For convenience, the results are presented for each lesion separately.

Capillary Hemangioma. Glycogen was present in varying amounts in the cytoplasm of the endothelial cells in each specimen of capillary hemangioma (Figs. 2 and 3). This was true not only in endothelium differentiated into capillary structures but also in small cords and groups of aligned, proliferated endothelium without lumina. Only when the content of glycogen was large did a few granules of glycogen appear free in the lumina.

Cavernous Hemangioma. Glycogen was present in slight amounts in the endothelium lining the irregular, dilated, blood channels in 3 of 4 specimens of cavernous hemangioma.

Arterial Hemangioma. Glycogen was demonstrated in varying amounts both in the cytoplasm of endothelial cells and in the smooth muscle coats of the arteries in both specimens of arterial hemangioma.

Sclerosing Hemangioma. The distribution of glycogen in sclerosing hemangioma is difficult to define as to particular cell types. As sclerosis progresses in a hemangioma, the intercapillary fibrous tissue increases, often with a hyaline change of the collagenous tissue, or a diffusely cellular picture is produced. The isolated or grouped cells, whether endothelial, fibroblastic, or histocytic, are difficult to differentiate. The only cells one can classify are endothelial cells lining formed capillaries and lipid-laden histocytes or "foam" cells. Considering all three cell types together, glycogen was found in varying amounts in the cellular cytoplasm in 18 of 23 specimens. The "foam" cells uncommonly contained intracytoplasmic glycogen. Their cytoplasm presented variable amounts of lipids in frozen sections. A definite but much smaller amount of

stainable lipid material was seen in paraffin sections. Often the hemosiderin and lipid granules in the histiocytes were colored pale to bright red by the periodic acid-leukofuchsin reaction in paraffin sections, and this staining reaction was unaffected by previous diastase digestion.

Dermatofibrosarcoma Protuberans. A large amount of glycogen was demonstrated in the cytoplasm of the neoplastic fibroblasts in 3 of 4 specimens of dermatofibrosarcoma protuberans (Fig. 1). The fibroblasts were arranged in small whorls or bundles, often around a central capillary. The endothelial lining of this central vessel sometimes contained a small amount of glycogen. Whether this tumor is related to the sclerosing hemangioma or is a primary neoplasm of fibroblasts is unknown.

Hemangiopericytoma. Glycogen was present in the endothelium of many of the small blood vessels and capillaries of the hemangiopericytoma, but was not found in the intervascular tumor cells proper (the neoplastic pericytes).

Kaposi's Sarcoma. The 2 specimens of Kaposi's sarcoma in the skin were characterized by numerous irregularly spaced capillaries between which there occurred proliferation of spindle cells and fibroblasts in association with hemorrhage and with scattered lymphocytes and histiocytes. Glycogen was abundant in the cytoplasm of the spindle cells and fibroblasts, and a lesser amount was found in the endothelium of the capillaries.

Normal Blood Vessels, Arrector Pili Muscle, and Fibroblasts of Human Skin. Thirty specimens of normal human skin showing arrector pili muscle, 5 specimens of dermal scar tissue, and 5 specimens of granulation tissue were examined histochemically for glycogen. The results are tabulated in Table II. Glycogen was demonstrated in varying amounts in the endothelium and smooth muscle of blood vessels and in the arrector pili muscle in 25 specimens of normal skin. The fibroblasts in the dermal collagenous connective tissue and in the dermal scar tissue rarely contained intracytoplasmic glycogen. In contrast, the proliferating fibroblasts in granulation tissue often presented intracytoplasmic glycogen, usually in small amounts.

Lubarsch[1] earlier demonstrated glycogen in "rhabdomyomas" and in many different types of epithelial neoplasms. This also is true of rhabdomyosarcomas, leiomyosarcomas, and carcinomas studied by me.[12] Ackerman, Knouff, and Hoster,[13] recently reported on the cytochemistry of normal and neoplastic cells in human lymph nodes. In normal lymph nodes, glycogen was demonstrated in the reticulum cells of the sinuses, in the reticular clasmatocytes, in the circulating neutrophils, and occasionally in the lymphocytes. With neoplastic in-

volvement of lymph nodes, glycogen was found frequently in the tumor cells in all specimens of metastatic carcinoma, frequently in the tumor cells of reticulum cell sarcoma, often in the modified lympho-

TABLE II

Glycogen in Normal Blood Vessels, Arrector Pili Muscle, and Fibroblasts of Human Skin

Anatomical structure	Glycogen
Endothelium of blood vessels	Often present in variable amounts
Smooth muscle of blood vessels (arterioles, small and medium arteries)	Often present in variable amounts
Arrector pili	Often present in variable amounts
Fibroblasts of normal connective tissue	Uncommon
Fibroblasts of healed scar tissue	Absent
Proliferating fibroblasts of granulation tissue	Often present in small amounts

cytes of lymphosarcoma and chronic lymphatic leukemia, and rarely in the Reed-Sternberg cell of Hodgkin's disease.

SUMMARY

The periodic acid-leukofuchsin reaction in tissue sections and in control serial sections previously digested in malt diastase provides a useful procedure for the histochemical demonstration of glycogen in the cytoplasm of tumor cells. Best's carmine method is equally satisfactory for the coloration of glycogen. These methods were applied to various vascular and related tumors—capillary hemangioma, cavernous hemangioma, arterial hemangioma, sclerosing hemangioma, dermatofibrosarcoma protuberans, hemangiopericytoma, and Kaposi's sarcoma. In addition, specimens of normal human skin and granulation tissue were studied.

A varying amount of glycogen occurred in the endothelial lining of the blood vessels in many of these tumors and in normal skin. A varying amount of glycogen also was present in the smooth muscle of normal arteries, of arrectores pilorum, and arteries in the arterial hemangioma. Glycogen was not found in the intervascular tumor cells of the hemangiopericytoma. It often occurred in the intervascular spindle cells (usually fibroblasts) forming the sclerosing hemangioma, dermatofibrosarcoma protuberans, and Kaposi's sarcoma. Fibroblasts of dermal collagenous tissue and of dermal scar tissue rarely contained intracytoplasmic glycogen, but proliferating fibroblasts of granulation tissue often presented small amounts.

It becomes apparent that intracytoplasmic glycogen is in no way peculiar to certain cells or to neoplasms arising from these cells.

Therefore, the presence or absence of intracytoplasmic glycogen cannot be considered a useful criterion for the histogenetic classification of neoplasms.

I am indebted to Dr. Fred W. Stewart, Memorial Center for Cancer and Allied Diseases, New York City, for providing many of the specimens examined in this study.

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DESCRIPTION OF PLATE

PLATE 159

The three illustrations were prepared from sections stained by the periodic acid-leukofuchsin method with an iron hematoxylin, picric acid counterstain.

FIG. 1. Dermatofibrosarcoma protuberans showing glycogen in the spindle tumor cells, grade 3. × 600.

FIG. 2. Capillary hemangioma showing diffusely distributed glycogen in the endothelium, grade 3. × 600.

FIG. 3. Diastase digested control on adjacent serial section of case shown in Figure 2. Glycogen has been removed completely. × 600.

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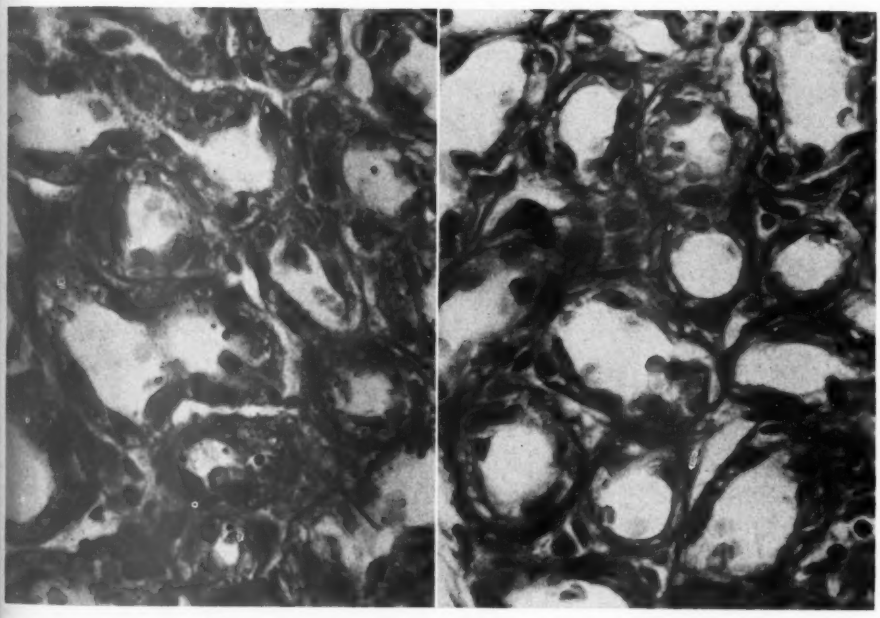

2 3

Bangle Glycogen in Vascular Neoplasms

UNUSUAL PATHOLOGIC MANIFESTATIONS OF DISSECTING AORTIC ANEURYSM
INCLUDING ONE EXAMPLE OF SO-CALLED INCOMPLETE RUPTURE*

WARREN C. HUNTER, M.D., and JAMES H. LIUM, M.D.
(From the Department of Pathology, University of Oregon Medical School, Portland, Ore.)

Shennan,[1] in 1934, published the first extensive review of dissecting aneurysm of the aorta, and since that time a voluminous literature on the subject has appeared. Recently, Gore and Seiwert[2] reviewed 85 fatal cases of dissecting aneurysm of the aorta and Gore[3] presented an excellent study of the pathogenesis of the lesion. These and many other readily available publications make a further review of the literature unnecessary at this time.

The purpose of this paper is to present four studies of unusual and interesting variants of dissecting aneurysm of the aorta, one of which raises the question as to the relationship of so-called incomplete rupture of the aorta and dissecting aneurysm.

Case 1 was an extensive, healed, dissecting aneurysm of the aorta that was diagnosed clinically. The false channel ended in a blind pouch, the communication between the true and false channels being situated well proximal to the actual end of the dissection. Furthermore, there was an associated and entirely separate arteriosclerotic aneurysm of the abdominal aorta which ruptured spontaneously resulting in the death of the patient approximately 4 years after the original dissecting lesion occurred.

In case 2 there were three small, separate points of communication between the true and false channels in the ascending aorta. The compressed but patent right coronary artery also traversed the dissected aortic layers. Another independent feature was coarctation of the aorta of adult type.

Case 3 had several features of unusual interest, including (a) two dissecting aneurysms of distinctly different ages, involving exactly the same part of the aorta; (b) organization rather than canalization of the thrombus associated with the older lesion; (c) healing for a short distance only at the proximal and distal ends of the communications of the earlier dissection with the aortic lumen; (d) origin of the final and very recent hemorrhage from a weak point at one margin of the healed aneurysm; (e) sufficient repair of the initial cleavage at other points to prevent completion of a total annular rent on the second occasion.

* Received for publication, May 15, 1952.

Case 4 exemplifies how, uncommonly, incomplete rupture or dissecting aortic aneurysm may occur in the presence of both the clinical and pathologic factors predisposing to the common form of rapidly fatal aneurysm. Yet, in this instance the aortic lesion was asymptomatic except as it might have accounted for an aortic murmur and it was discovered in a routine necropsy.

CASE 1

The patient (305-ma-50), a 67-year-old white male, was first admitted to Multnomah County Hospital on September 5, 1946, with the chief complaint of abdominal pain. He described the sudden onset on September 2 of severe pain at the lower lumbar level, which radiated down the right leg and into the lower abdomen from both sides. He had noted that the right leg (left leg absent from mid-thigh amputation in 1926 following a compound fracture complicated by infection) was numb, cold, white, and painful until September 4, when he again became asymptomatic. Chest pain, cough, or hemoptysis were denied. The favored clinical impression at this time was dissecting abdominal aneurysm. On September 9 symptoms suggesting obstruction of the colon necessitated laparotomy, and palpation of an aneurysm of the distal aorta was reported. No tumor or other obstruction was found. Recovery was uneventful, and he was discharged from the hospital on December 19.

The interval history revealed several hospital entrances, none of which have direct bearing on the present discussion. The recorded blood pressure varied from a high of 205/125 to a low of 180/110 mm. of Hg. On one of these admissions a diagnosis of hypertensive cardiovascular disease was established, the heart being described as enlarged and having an aortic configuration.

The final admission to Multnomah County Hospital was on October 17, 1950. On October 16 he had noted a sharp, shooting pain originating in the left flank posteriorly which radiated to the left lower quadrant of the abdomen, but not to the right leg or the stump of the left. On the evening of October 17 he experienced a similar pain which was severe enough to cause temporary loss of consciousness. Physical examination revealed a pulse rate of 92 per minute and a blood pressure of 74/56 (?). The patient was described as pale, thirsty, and drowsy, and had cold skin. There was slight dyspnea and occasional complaint of abdominal pain. Examination revealed a relaxed abdomen with some voluntary muscle spasm and guarding to deep palpation, especially in the left lower quadrant. The left flank appeared distended. The bowel sounds were absent. No viscera were palpable. The clinical impression was retroperitoneal hematoma, probably secondary to the extension of a dissecting aneurysm. Roentgenologic examination of the chest and abdomen on October 18 suggested hypertrophy of the left ventricle and a markedly tortuous and elongated aorta, particularly in the descending thoracic portion. There were increased soft tissue densities throughout the upper left abdominal cavity with obliteration of the psoas and kidney shadows. The left hemidiaphragm was elevated. Despite supportive therapy death occurred suddenly on October 19, 1950.

Necropsy Findings

At necropsy the heart weighed 580 gm., the increased weight being predominantly due to hypertrophy of the left ventricle. There was moderate subendocardial myofibrosis, but no recent infarction. There were no mural thrombi. The coronary arteries showed only moderate arteriosclerotic narrowing.

The aorta displayed marked atheromatous changes throughout its length, as did also the proximal portions of the common iliac arteries. The proximal 9 cm. of the aorta was the seat of fusiform dilatation and increased tortuosity. Approximately 9 cm. distal to the aortic valve ring, the aorta divided into two distinct channels, both of which were lined by endothelium, giving the aorta a "double barreled" appearance throughout most of its length (Fig. 1). The ostium of the false channel was immediately distal to that of the true channel, arising on the left lateroposterior portion of the aortic wall. The false channel followed diagonally across the posterior portion of the transverse arch, continued down the right lateroposterior portion of the aortic wall and ended in a blind pouch approximately 9 cm. proximal to the bifurcation. Multiple major vessels arose from the false channel. Eight cm. distal to the origin of the false channel there was an irregular, smooth, 1.2 by 1.0 cm. perforation of the wall separating the true and false channels, and constituting the distal communication between them (Fig. 1,e). There was a large, arteriosclerotic, saccular aneurysm of the lower abdominal aorta, the inferior margin of which was approximately 1.0 cm. proximal to the aortic bifurcation. The sac was almost completely filled by an old, laminated, mural thrombus, only a narrow passageway remaining through which blood was reaching the lower extremities. A small area on the superior, left, lateral portion of the wall of the aneurysmal sac remained unprotected, and at this point a rupture of the aorta was identified as the source of the massive retroperitoneal hemorrhage filling the left side of the abdominal cavity. This was regarded as the immediate cause of death.

Microscopic Observations. The dissected part of the aorta exhibited arteriosclerotic changes involving both the true and endothelialized false channel. The outer wall of the false channel was somewhat thinner than the septal wall between the two channels. A few focal cystic spaces in the media compatible with so-called cystic medionecrosis were identified. There was no evidence of syphilitic aortitis.

Discussion: Case 1

Reports of healed dissecting aneurysm of the aorta, so-called double aortas, are fairly frequent in the literature. One such case with a survival of 9 years following the initial dissection has been described recently.[4] Jones and Langley[5] have recently re-emphasized that entry back into the aorta or one of its branches seems almost a prerequisite for survival, although this does not absolutely prevent external rupture. The most common site of re-entrance seems to be the distal aorta near the bifurcation or the iliac arteries. The re-entrance of blood

from the distal end of the dissected sac into the lumen of the vessel permits circulation through the new channel and is the most important determining factor in the process which leads to healing and production of a separate channel.[1]

In our case 1, the point of re-entry into the true channel was considerably proximal to the end of the dissection. The false channel, devoid of thrombus, endothelialized, and the seat of sclerotic plaques, ended in a blind pouch. Numerous reports of major vessels arising from the false channel are to be found in the literature, and in this instance such was the case. The apparent, highly placed, vascular occlusion of the blood supply to the right leg at the time of the initial dissection is of interest. In acute dissections, East[6] emphasized pathognomonic changes in the circulation of the legs due to obstruction of the aorta, which may be helpful in diagnosis. More recently, Shucksmith and MacPherson[7] reported the details of a fatal case of dissecting aneurysm of the aorta simulating embolism at the aortic bifurcation, and stated that the difficulty in diagnosis of the vascular cause of sudden pain and paralysis in a limb is the element of arterial spasm which may occur in embolism and in dissecting aneurysm. At operation, the reported visualization of an aneurysm was undoubtedly a description of the associated, independent, saccular, arteriosclerotic aneurysm of the abdominal aorta. The fact that the spontaneous rupture occurred through an area unprotected by the mural thrombus is of interest.

CASE 2*

The patient (435-a-50), a 46-year-old white female, was first seen in January, 1948, complaining of pain in the left arm and back. There was a long-standing history of hypertension, and the recorded blood pressure on this visit was 212/126 mm. of Hg. There was moderate enlargement of the left ventricle. She was admitted to the hospital on January 28, complaining of severe pain of sudden onset in the upper precordial region, radiating through to the back and down into both flanks.

The pulse rate was rapid, and the blood pressure was 172/112. The next day it was 154/68, and she developed systolic and diastolic murmurs over the entire precordium. The cardiac murmurs gradually diminished. She was again hospitalized in March, complaining of dyspnea and chest pain; the blood pressure was 160/70. There was cardiac enlargement and râles were heard in the right lung base. Digitalization brought considerable improvement in symptoms. In June and December of the same year there were episodes of congestive heart failure which responded nicely to oxygen and mercurial diuretic therapy. The patient was followed in the office until March, 1950, when she again had congestive heart failure. The blood pressure was 180/0. She was placed on a low-sodium diet, treated with oxygen, and in a few days was asymptomatic. She was again hospitalized in May and August of 1950 for severe congestive failure. At the last admission there were moist râles in both lung bases. The heart was greatly enlarged, and there was a loud to and fro murmur over the entire precordium. The blood pressure was 180/0. There was 2 plus pitting edema

* We are indebted to Dr. William A. Burget of the Astoria Clinic, Astoria, Oregon, for the very complete clinical information.

of the legs and ankles associated with cyanosis of the lips, nail beds, and mucous membranes. Death occurred suddenly on August 7, 1950.

Necropsy Findings

Necropsy was performed in another city, and only certain tissues were submitted to us.

The bulk of the specimen, weighing 730 gm., consisted of the heart and entire aorta, together with about 7 cm. of each common iliac artery. The aorta had been opened previously throughout its length, and the left ventricle also was opened. There was dilatation of both cardiac ventricles. Hypertrophy of the left ventricle was marked. Scattered in the myocardium of the posterior portion of the left ventricle were tiny fibrotic patches; at the apex the myocardium was extensively replaced by whitish fibrous tissue. There was some bulging of the apical interventricular septum into the right ventricle. The leaflets of the aortic valve showed slight fenestration and atheromatous mottling. The circumflex and anterior interventricular branches of the left coronary artery and its main trunk were patent and showed only minimal focal arteriosclerotic change. The right coronary artery was patent except in the area where it traversed the dissecting part of the aortic layers.

Between the pulmonary artery and the right atrial appendage there was a saccular cavity measuring about 10 by 8 by 5 cm. (Fig. 2). This was a portion of a sac produced by the dissection of the aortic wall throughout its length. One gained the impression that the sac of the dissecting aneurysm in the ascending aorta could very readily have produced compression of the pulmonary conus and first part of the pulmonary artery. Certainly, it had obliterated the right atrioventricular groove. In this portion of the sac, there were three communications between the sac and the proper intimal surface of the aorta just above the aortic cusps. Two of these occurred close together, and were 3 and 5 mm., respectively, in diameter. These fenestrations were located just above the anterior aortic sinus, and just beneath them was the slit-like opening of the right coronary artery, measuring about 4 by 1 mm. Around all but the inferior margin, the ostium was surrounded by a raised ring of intimal sclerosis. The third opening, 5 mm. in diameter, was located just behind the right posterior aortic cusp.

The true intima of the ascending aorta was almost entirely free from sclerosis. This was in striking contrast to the remainder of the vessel. Following the dissection distally, the presence of an aortic coarctation of the adult type, situated immediately proximal to the aortic end of the ductus arteriosus, apparently was responsible for the fact that the

dissection in the thoracic aorta had stopped on the left side. The descending aorta was not a complete double tube, 2 to 2.5 cm. of the anterior wall having failed to split anywhere. In the ascending portion of the sac and more often in the descending aorta, on both sides of the inner tube and bridging between the two segments of the wall, were a number of thin, white, shiny cords representing remnants of vessels. None of the larger branches of the abdominal aorta lay in the line of cleavage and none was appreciably narrowed. The dissection stopped at the bifurcation of the abdominal aorta on the right side, but continued along the left common iliac artery for a distance of 5 cm. The common iliac arteries were fully as sclerotic as the aorta.

Microscopic Observations. A large block from the abdominal aorta, including the complete circumference of the vessel, showed thickening, hyalinization and fatty deposits in the true intima. This was far thicker than the pseudo-intima that had formed on the two surfaces of the false channel. Cells resembling endothelium were found only occasionally, the immediate lining being degenerated and hyalinized media, without thrombus formation over it. Focal areas of calcification were present. Consistently lacking in the various sections of the aorta was evidence of medial degeneration with cyst formation, such as one often observes in dissecting aneurysm. Transverse sections from the segment of the right coronary artery as it passed through the base of the false sac revealed a patent lumen, elliptic rather than round. There was some intimal thickening with deposits of lipid-laden cells. The adventitia was wholly lacking, as would be expected for the intramural part of a coronary vessel. Of interest was the fact that there were several good-sized areas of degeneration and hyalinization of the media of the coronary artery. There was no evidence of thrombosis, old or recent.

Discussion: Case 2

It is well known that dissecting aneurysms extending to the root of the aorta commonly rupture into the pericardial sac with resultant cardiac tamponade. Perhaps the multiple stomata in the ascending aorta may have prevented such a catastrophe in this case, especially if they formed at the time of the original dissection. These communications appear to be of relatively long standing, and may possibly account for the to and fro murmurs heard clinically. A second interesting feature of this case was the traversing of the aneurysmal sac by the right coronary artery. Although the vessel was not occluded, the lumen appears flattened microscopically, and thus coronary insufficiency may well have existed. The number of reported cases of coronary artery involvement in dissecting aneurysm is small. Oram and

Holt[8] recently reviewed the ways in which the coronary blood supply can be rendered inadequate by dissecting aneurysm.

CASE 3

A male (A-36-52 P. San.), 60 years of age, had had known hypertension and repeated episodes of heart failure for the past several years; 9 months prior to death he suffered a right hemiplegia. A few days prior to the first hospital admission on January 31, 1952, there was complaint of acute nausea, vomiting, and headache, together with some pain in the right upper quadrant and back. The severity of the pain or changes in its location are not noted in the history. In view of the roentgenologic finding of duodenal deformity, suggesting peptic ulcer, ulcer management was instituted and after 3 days he was discharged. The blood pressure recording was 220/120 mm. of Hg.

The final admission, on April 19, 1952, preceded death by only 3 hours. Starting with dizziness and numbness of the left side, he quickly became comatose and died. The blood pressure was 280/120.

Necropsy Findings

At necropsy, the following were found to be of interest: generalized arteriosclerosis; renal and pancreatic arteriolosclerosis; massive acute pontine hemorrhage, extending into all ventricles of the brain; hypertrophy of the heart (790 gm.); renal adenoma (3 cm. in diameter); nothing indicative of duodenal ulcer, active or healed, and two dissecting aneurysms of the aorta, to be described in detail.

Gross Appearance of Aorta. Viewed in the posterior-anterior direction, the opened descending portion of the aorta at once revealed an extensive, fresh, incompletely annular splitting of the vessel through the media, with both fluid and newly clotted blood in the space and many circularly disposed parallel ridges on the inner aspect of the outer segment. Rupture through the adventitia or backward into the original lumen of the vessel could not be demonstrated. Superiorly, the new cleavage fell a trifle short of the right subclavian artery; inferiorly, it ended at the level of the renal arteries. Palpation of the anterior wall elicited a doughy sensation in places and induration in other parts of this wall, with quite evident cessation of the recent tear.

Located in the anterior aortic wall, beginning 2.3 cm. inferior to the ostium of the right subclavian artery, was an abnormal opening 1.5 by 1.5 cm. and 1 cm. in depth, shallow and saucer-like on one side and with a smooth and overhanging margin on the other. The base of the shallow portion was glistening, that of the deeper part dull and rough. Five mm. to the right of the opening just described was another, 0.7 by 0.3 cm., leading downward for 0.7 cm. into a thrombus, and like the other defect, possessing a shiny intimal margin. Both gave the impression of being healed defects. A transverse section of the vessel wall at this level revealed an apparent thin band of intima between

the stomata, forming a partial septum which ended in a firm reddish brown thrombus having a thickness of 1 cm. and a breadth of at least 2 cm. Each opening ended blindly and did not reappear elsewhere in the clot. Superiorly, the thrombus gradually became smaller and stopped within the media just short of the ostium of the subclavian artery.

Two additional abnormal stomata were noted in the right anterior wall, 13 cm. distal to those just described. Separating these was a smooth intimal septum 1 mm. broad. The openings measured 5 by 2 mm. and 5 by 5 mm. and presented glistening margins, quite like the surrounding intima. A transverse slice at this level indicated plainly that both openings, after enlarging very slightly and finally lying 6 mm. apart, were mere burrows into a firm brownish thrombus, ending blindly after attaining a depth of only 6 mm.

Cuts at intervals of 5 to 7 mm. throughout the length of the aorta disclosed some variability in the size, consistency, and color of the thrombus (Fig. 3). Both above and below the superior stomata the thrombus was firm, reddish brown, and relatively thin and flat, but within a few centimeters it became appreciably thicker, soft and black centrally, and chocolate brown peripherally. Finally, nearing the inferior openings, the clot thinned out, and became elongated and light brown to almost white.

Immediately around much of the thrombus one could make out firm and white wedge-shaped areas, appearing like connective tissue. However, in one area in the right lateral wall, at a point where the largest and thickest portion of the thrombus lay, there appeared to be a deficiency in the tissue and this was thought to be the source of the new hemorrhage which almost encircled the vessel. The inner wall of the new dissection consisted of intima containing scattered yellow or white plaques, while the outer layer was composed of the adventitia and at least a part of the media.

Microscopic Observations. The base of the more shallow portion of the larger, superiorly located defect consisted in part of abundant young connective tissue in the location of the intima and then, as the margin of the defect was approached, of organizing thrombus. Even here it was apparent that the media had been split, as evidenced by a thin zone of relatively young vascular connective tissue, separating the media into two parts. Still further outward there was an absence of elastic fibrils in parts of the middle third of the media and a bluish staining reaction rather than an acidophilic one, constituting one of the medial lesions predisposing to dissection. The smaller of the superior openings was, in part, separated from the larger one by a bit of

turned-over media and intima bound to a thrombus, at this point unorganized, while the remainder of the opening was bordered by thrombus alone. In contrast, the small openings, 13 cm. inferiorly, were both surrounded by abundant young connective tissue, circularly disposed in the manner of a vessel wall, but devoid of elastic fibers, and directly continuous with the thickened and hyperplastic intima and with much less cellular but vascular connective tissue filling the old dissection. Save for the area of poor organization at the periphery of the largest part of the clot within the old dissection, a very appreciable amount of connective tissue filled the defect and bound the media firmly. The center of the larger parts of the intramural thrombus was undergoing softening. Along the right border of the weakly organized thrombus, approximately midway between the superior and inferior stomata, there was microscopic proof of a direct connection between the old and new dissections. The thrombus in the latter was made up of coarse strands of fibrin with enmeshed leukocytes and erythrocytes, in contrast to the amorphous structure of the older thrombus.

Discussion: Case 3

While it is by no means unusual to encounter healed dissecting aortic aneurysms, commonly these are of the "double-barreled" type, in which one or more defects provide inflow and outflow channels. Usually too, under this circumstance, the false channel is fully open, rather than occluded by a thrombus. It is logical to believe that in this example the more caudal communications either did not exist at the time of original dissection, or if so, that they were always very small and prevented or slowed the egress of blood until a thrombus formed throughout the length of the cleft.

In this example openings existed at each end of the older lesion but quickly terminated in an organizing thrombus superiorly and as structures lined with connective tissue inferiorly. The remainder of the original dissection failed to canalize.

Whenever the thrombus spread widely and thinly complete organization occurred, cementing the media once more, but in those areas where it was thick and bulky healing was found only at the periphery and the union was a weak one. There is positive microscopic proof that the terminal dissection began along one border of the poorly organized original thrombus and spread rapidly to the point of encircling the remainder of the aorta. One wonders whether, with a very probable elevation of systolic blood pressure after the rapidly fatal intracranial hemorrhage began, additional strain was placed on the aorta already weakened by incomplete walling-off of the first dissection and with the

predisposing factor of focal medial degeneration in existence, and thus initiated the final dissection, which was so recent that the patient's history gave no indication of its presence.

Although absolute proof is lacking, it is very probable that the first dissection took place approximately 2½ months prior to death, as evidenced by the complaint of right upper abdominal and back pain, thought to have been due to peptic ulcer, no evidence of which was found at necropsy.

The case is of further importance in that it affords an explanation for the peculiar minor cleavage of the aorta in case 4. After about 2½ months, we see microscopic evidence of advanced healing of the unquestionably torn intima and media, and of the underlying thrombus. If only the more superior defects had developed in connection with a blindly ending dissection as in case 4, and if the patient lived for an appreciable period of time, is it not conceivable that a very firm union of the base of the cleft might have occurred, the connective tissue have been much reduced by compression from the tension within the artery, a new endothelium formed, and a picture like that of the defect in case 4 might have resulted? Is it not possible for very short dissections of the aorta to occur? If this concept is correct, some of the lesions described in the literature as "incomplete rupture" of the aorta actually may be dissecting aneurysms?

CASE 4

The patient (M-155-6-40), was a male, 71 years of age. Five years prior to death he became dyspneic on exertion and for the past 10 years noted occasional frontal headache and episodes of vertigo. The blood pressure on the first hospital admission, 3 years before death, was 180/96 mm. of Hg, the apex beat was in the sixth left interspace, and a blowing systolic murmur was heard there. The second and last admission, 1 month before death, was for interscapular and inferior sternal pain, coming on during the afternoon and persisting through the night. The pain was not brought on by exertion; rather, motion brought relief. Dyspnea was not a complaint. The blood pressure was 180/100, with the heart apparently unchanged in size, but in addition to the apical systolic murmur there was now a systolic aortic murmur, transmitted upward. For years there had been marked urinary frequency. To palpation the prostate was indurated and nodular medially. The peripheral arteries were sclerotic.

The clinical diagnoses were: probable coronary occlusion; possible dissecting aneurysm; osteo-arthritis of spine; probable carcinoma of the prostate with metastasis. Both blood and spinal fluid gave a negative serologic reaction for syphilis.

Necropsy Findings

At necropsy, the following observations were made: advanced adenocarcinoma of the prostate with metastasis to the lumbar vertebrae; osteo-arthritis of the thoracic spine; generalized arteriosclerosis; terminal bronchopneumonia; cardiac hypertrophy (540 gm.).

Aortic Lesion. Of no apparent importance as a cause of death, but of great pathologic interest, was the ascending aorta. The aortic valve measured approximately 8 cm. in circumference, with the lumen increasing to 11 cm. immediately above the attachment of the cusps. There was a distinct pocket in the vessel wall, having one end lying superior and a little anterior to the ostium of the left coronary artery. The opposite terminus was well above and a little to the left of the right coronary ostium, wholly within the left half of the ascending aorta (Fig. 4). The free margin of the pocket had a smooth surface, averaging 2 mm. in thickness. This abnormal structure resembled a thick and very large semilunar valve cusp. When the opened aorta was re-approximated and the pocket viewed from above, no appreciable protrusion inward at this point could be noted; rather it reminded one of a slightly open and sagging coat pocket. Since there was no localized protrusion of the outer wall of the vessel at this level, the structure bore no resemblance to a saccular aneurysm. The intimal surface, both over the inner flap and within the cavity itself and like that of the ascending aortic intima elsewhere, was studded with whitish plaques, without ulceration or calcification. The lining of the pouch was glistening throughout. Specifically, there was no suggestion of the linear wrinkling of syphilis.

The defect had a maximum depth of 1.5 cm. and a thickness of 2 to 3 mm. In comparison, the outer segment of the aortic wall measured 3 mm., not including the adventitia. The left coronary artery pierced the aorta immediately inferior to the base of the pouch but was not narrowed in any way.

Microscopic Description. Multiple sections from a block cut to include the full thickness of the aortic wall both superior and also inferior to the defect, together with all of the inner flap, were stained with hematoxylin and eosin, van Gieson's stain, and for elastica by the Weigert and Verhoeff technics.

While both the inner and the outer aspects of the flap consisted of acellular hyalin-like material, save for some bits of calcium at the free margin, it was evident that the center was media because of the presence of as many as thirty layers of elastic fibers in parallel arrangement, still evident despite fragmentation and separation by hyalin. At the base of the pouch, the elastic fibers were much more compactly arranged than elsewhere and curved upward into the segment of the aortic media and also directly and without interruption into the flap itself, as previously mentioned. The number of elastic fibrils in the outer part of the vessel wall numbered from fifty to sixty and exhibited less fragmentation and hyaline degeneration than those in the flap.

Replacing the original media throughout the pocket was dense hyaline tissue. There was no thrombus formation anywhere.

Discussion: Case 4

The microscopic structure of the aortic wall at the level of the defect and of the adjacent portion of the vessel inferiorly and superiorly indicated convincingly the existence of a cleft through the media. True intima existed over the aspect of the flap bordering the main aortic lumen; false intima, consisting of hyalin alone, lined the walls of the pocket. The cleavage of the media evidently occurred somewhat toward the inner third of this coat, if one may judge by the greater number of parallel fibrils in the outer segment. The presence of layers of elastic fibers within the flap itself, and especially the fact that these bridged across the base of the defect to become continuous with those in the outer segment of the aorta, are regarded as evidence of cleavage of rather short length and of mild degree in order to save these fibrils intact. It is remarkable that the split was so sharply limited, that it failed to extend further at any time, healed completely, and that no indication of old hemorrhage existed in its vicinity. The many foci of degeneration, absence of medial elastica, and the frequent small clefts in this coat are features often observed in aortas with dissecting aneurysm. On the other hand, the focal softening, fraying, and edema about the vasa vasorum, commonly found as predisposing lesions in dissecting aneurysm, were lacking. There were no endarteritis or perivascular collections of leukocytes anywhere.

The location and even the size of the open end of the defect are not unlike the features of intimal rupture observed in acutely developing dissection and also in some healed dissecting aneurysms such as our case 3, but at this point the resemblance ceases. If the dissection in case 3 had been small and short, it is not difficult to imagine that had the thrombus within it healed in time, the resulting structure might have closely resembled that of this case.

The location of the defect is hardly in keeping with a supernumerary aortic valve cusp and the positive microscopic proof of medial as well as intimal structure, together with direct continuity with the remainder of the media, rule out supernumerary valve. Against the possibility of origin by hemorrhage and excavation of an atheromatous intimal ulcer is again the fact that not only intima but much media share in the composition of the flap.

The clinical features of the case throw no real light on when the dissection took place unless the systolic aortic murmur noted on the last admission can be ascribed to the aneurysm. If so, the absence of

this sound on the prior admission 3 years earlier might indicate that the damage occurred some time in that interval. Peery[9] and also Spicknall and Binford[10] have observed the occurrence of aortic murmur in the presence of "incomplete rupture of the aorta," which Peery regarded as closely akin to dissecting aneurysm.

General Discussion of So-Called Incomplete Rupture of the Aorta

Peery,[9] in 1942, writing mainly from the clinical point of view, described the characteristics of both recent and healed incomplete rupture of the aorta. He did not mention the detailed structure of the margins and the remainder of the vessel wall other than the presence or absence of medial degeneration or evidences of syphilis, with two exceptions. In his case 3 it was observed that the base of the tear was crossed by several bands of torn media covered over with scar tissue, and again in case 7 sections taken across the tear displayed degeneration of muscle and elastic tissue of the media, and replacement by a mucoid soft fibrous tissue, together with a few mononuclear cells in the base of the defect. Without stating that he was writing specifically of personally studied healed or healing examples, Peery described the microscopic character of the lesion in incomplete rupture of the aorta as tears showing an abrupt interruption of the media involving approximately the inner two-thirds of that coat, breaking and fraying of the elastica, an intima that was usually thickened and hyaline at the base of the tears, and scar tissue in the disrupted media. The media might appear normal a few millimeters back from the edges of the tear. Exceptionally, in Peery's series, mucoid changes were noted in the media, but since they were found only at or near the tear, they were regarded as degenerative changes following the tear. Again apparently generalizing, older incomplete tears were described by Peery as occurring usually in the ascending portion of the aortic arch, at or near the end of the commissures, and directed mostly transversely, occasionally obliquely, and rarely longitudinally. Size varied considerably; most often the tears were 0.5 to 2 cm. in length. Owing to the gaping which occurs, they usually were elliptic, with sharp and clearly defined edges. The lips of the tear usually were 3 to 6 mm. apart with a slightly outward bulging of less than 1 cm. between the lips. The base was commonly smooth, white and glistening, and might show slight trabeculation.

Between 1935 and 1942, Peery[9] saw 6 instances of incomplete rupture without dissecting aneurysm; during the same years 2 cases of dissecting aneurysm were studied in which the clinical record strongly

suggested that the process occurred in two stages, and also several incomplete tears in cases of death due to dissecting aneurysm, giving a total of 8 examples among 1,600 necropsies. Three additional examples were encountered in a review of the necropsy records. There was a high incidence of hypertension on the services from which all of these cases were derived. Mention was made that if one judges the incidence of incomplete rupture of the aorta from the literature it would have to be considered a rare lesion, but that Gallavardin and Gravier[11] and Maresch[12] indicated that it is frequently overlooked at necropsy. Peery was of the opinion that in many instances complete rupture takes place through the base of an old tear, thus obliterating evidence of its previous state. Five of his examples were healed tears, encountered incidentally at post-mortem examination. There are many points of similarity between these cases and our case 4. Two additional patients had healed incomplete ruptures situated close enough to the aortic valve to produce aortic insufficiency. Finally, there were 3 examples of dissecting aneurysm which probably followed incomplete tears, starting 17 months, 7 months, and 4 days, respectively, before the final episode.

Peery[9] viewed incomplete aortic rupture as closely akin to dissecting aneurysm and as the condition which exists after a tear has occurred in the vessel wall but before dissection has begun. It appears to us that this is a fine distinction and that it would be equally logical to term the process as primarily a dissection of greater or lesser degree and to drop the term incomplete rupture. Are dissecting aneurysms of the types we have described in our first 3 cases not examples of incomplete rupture of the vessel? Yet they are lesions which anyone would unhesitatingly call dissecting aneurysms. Conversely, is there not clear-cut evidence of cleavage of the aortic media in our case 4? Why, then, term the latter an incomplete rupture and the others dissecting aneurysms? Our objection to the term "incomplete rupture" is that it introduces another name, possibly connoting to the reader a different etiology and a different pathologic process for what seems to be essentially dissecting aneurysm, the difference being one of degree rather than of kind.

From Bolivia, Wenger,[13] in 1943, reported observations on 2 cases of medionecrosis cystica idiopathica with rupture of the inner coats of the ascending aorta, not followed by dissecting aneurysm in the usually understood sense. The points of rupture were filled in by connective tissue, and elastic tissue had regenerated at the site of rupture in one case but in the other only connective tissue filled the defect. In one instance a complete rupture occurred later in the vicinity of the first

and probably began about 5 hours after a cerebral hemorrhage. Thus it is reminiscent of our case 3. Macroscopically, neither of Wenger's examples resembles our case 4, for one was ulcer-like and the other a curved groove. Histologically, one of Wenger's examples displayed a torn stump of media on one side of the tear and a streamer of intima on the other, a base nearly filled in by connective tissue and remnants of old media below this; there was no real dissection. The photomicrograph of the other case depicted a thin crescentic scar starting at the groove in the intima, running at a right angle well into the media, and then turning to one side for a short distance within this coat. One of his subjects had hypertension.

The study of Fisher and Salmons,[14] in 1950, concerned a single example of healing of an incomplete spontaneous rupture of the ascending aortic wall which was discovered at necropsy. The lesion encircled the vessel about 2.5 cm. above the sinuses of Valsalva and there was dissection of the intima and media for a distance of 2.5 cm. in each direction from the rupture. Endothelialization appeared to have occurred, with three or four dense fibrous strands between the combined intima and media and an unchanged adventitia. Neither cystic nor necrotic changes were observed in the aortic media. The patient was hypertensive and had died in congestive heart failure. These authors included a brief discussion of the etiology and pathogenesis of incomplete partial rupture of the aorta, which may be summed up by saying that little or nothing is known definitely about either.

Another example of partial rupture and dissection of the aorta was depicted grossly and microscopically by McMillan[15] in his study of an instance of diffuse granulomatous aortitis with giant cells. The defect was located in the ascending aorta, extended in a superior-inferior direction, had irregular margins, and microscopically the lips were rounded and contained a few imperfect elastic fibers. The margins were under-cut by a lateral dissection, extending for only 1 to 2 mm. on either side. The granulomatous reaction occurring elsewhere in the aorta was present in the area of rupture but in less severe form. There was no mention as to whether the author believed that the aortitis had a causal relationship to the tear.

Summary

Four case reports of unusual and interesting variants of dissecting aneurysm of the aorta are presented. Case 1 illustrates that re-entrance of the dissection into the true channel can take place at some distance from the end of the sac, and yet the blind pouch may heal without thrombosis. This case is of additional interest because it re-

mained for an independent arteriosclerotic aneurysm of the abdominal aorta to rupture and cause death.

In the second example, the multiple stomata between the true and false channels in the ascending portion of the aorta apparently formed early enough to prevent rupture into the pericardial sac, and also may have accounted for the murmurs heard clinically. The possibility of coronary insufficiency was suggested by the fact that the right coronary artery traversed the proximal portion of the sac.

Case 3 presented a well defined, old dissection filled with thrombus despite recommunication with the main aortic lumen. At the point where the thrombus was the largest and organization the poorest, there occurred a new rupture and cleavage that finished encirclement of the aorta at the same level and also distal to the old dissection.

The lesion in case 4 was of minor clinical importance, but nevertheless presented positive evidence of a split through the media with complete healing. We regard this as a small dissecting aneurysm, not an incomplete rupture, although some opinions in the literature would support the concept of incomplete rupture with healing. The aortic murmur heard during life may have been related to this pocket.

In all instances the usual concomitant clinical features of hypertension and cardiac hypertrophy were present. In 3 of the 4 cases microscopic evidence of medial degeneration existed.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 160

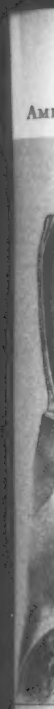
FIG. 1. Case 1 (305-ma-50). Reduced from a full-size drawing illustrating the dissecting aneurysm. (a) Cavity of left ventricle. (b) Anterior view of proximal end of old dissection, arrow to left passing along the course of the main aortic lumen (c). (d) False channel in ascending aorta, arch, and descending aorta. (In order to depict the relationships of the true and false channels at this level it was necessary to divide the septum between them and pull the wall to one side.) (e) Communication between the true and false channels far short of the distal end of the blind lower portion of the dissection shown at (f). (g) Mural thrombus in arteriosclerotic aneurysm. (h) Point of rupture of arteriosclerotic aneurysm leading to fatal hemorrhage. (i) Aortic bifurcation. (j) Small mural thrombus in true channel of aorta.

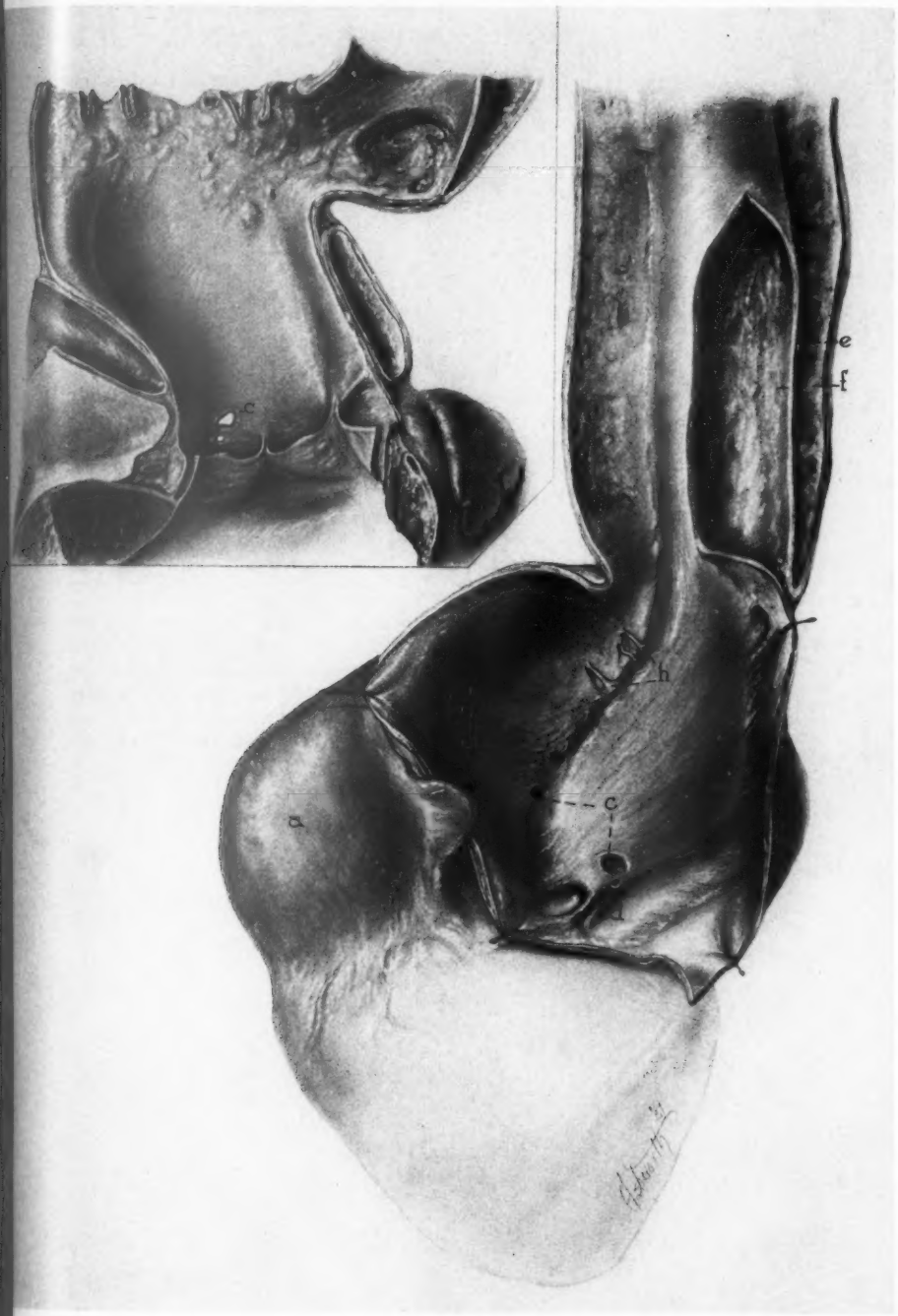
1

Hunter and Lium Dissecting Aortic Aneurysm

PLATE 161

FIG. 2. Case 2 (435-a-50). Reduced from a full-size drawing of the dissecting aneurysm. (a) Right atrial appendage. (b) Aneurysmal dilatation of the false channel. (c) Communications between the true and false channels. (d) Right coronary artery traversing aneurysmal sac. (e) False channel. (f) True channel. (g) Coarctation of aorta. (h) Small arterial branches traversing aneurysmal sac.





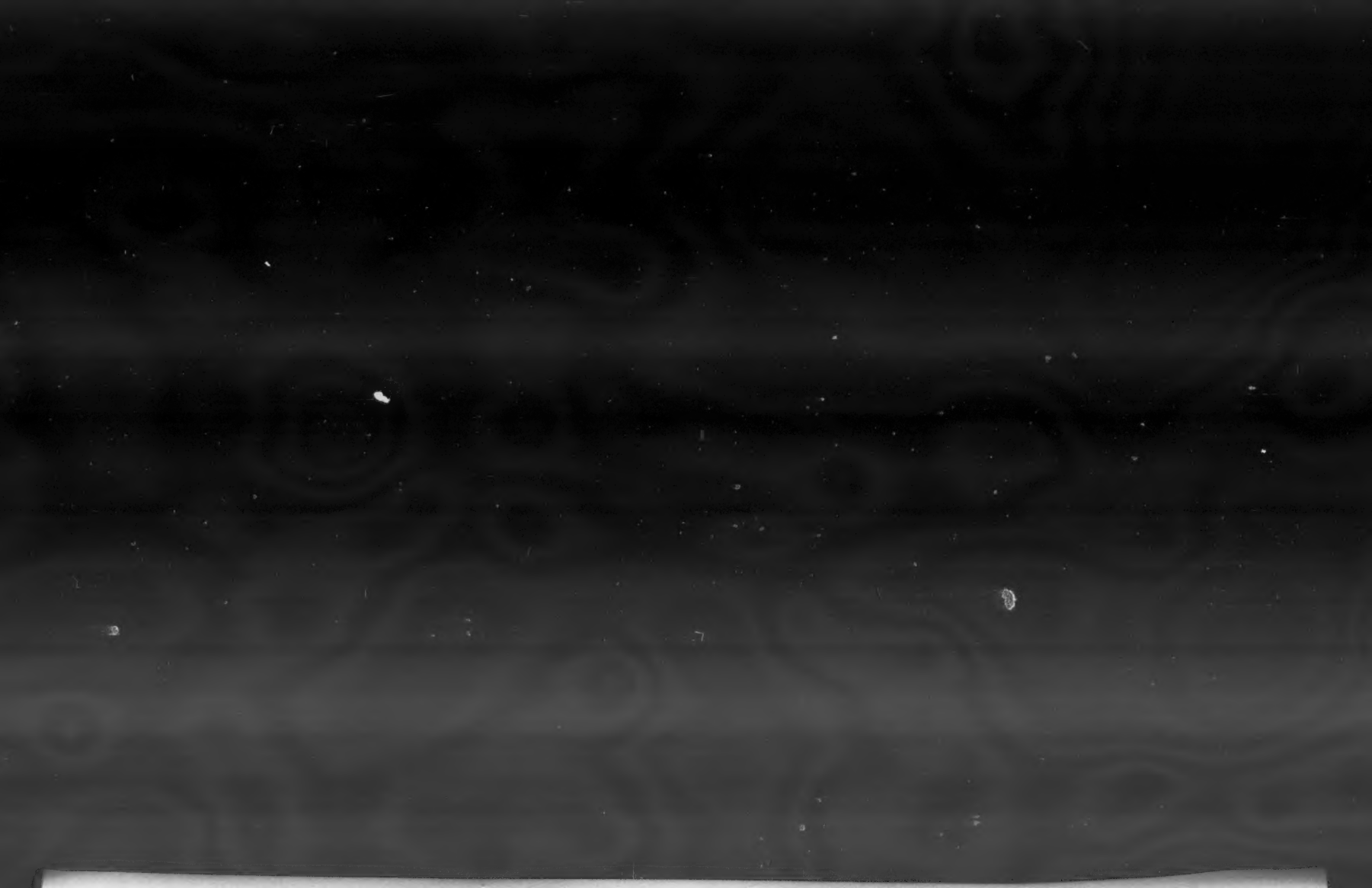
2

Hunter and Lium Dissecting Aortic Aneurysm

PLATE 162

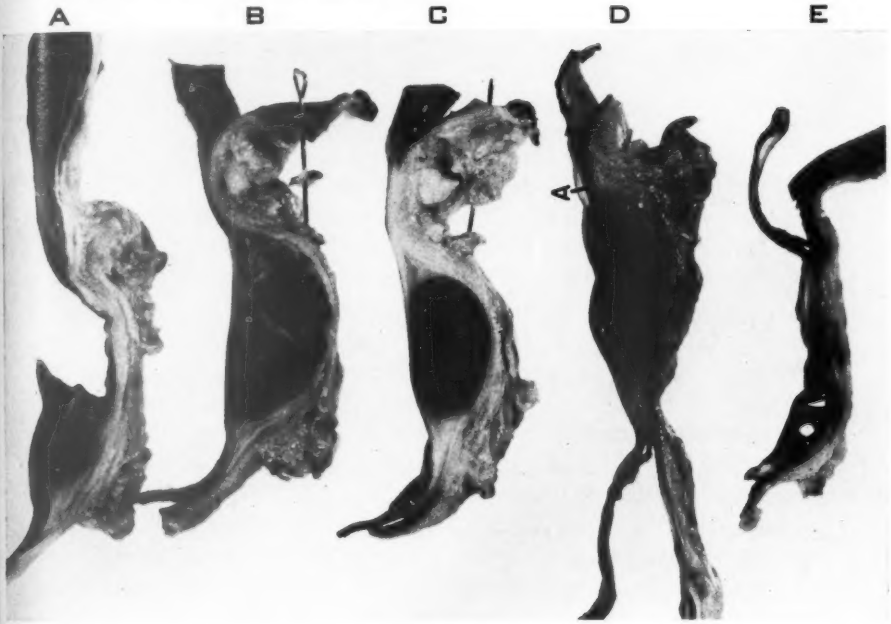
FIG. 3. Case 3 (A-36-52 P. San.). Reduced slightly from a natural size photograph of transversely cut segments of the aorta. A. Larger of the two superior defects with thrombotic base. B, C, and D. Selected areas from points between the upper and lower communications of the older aneurysm with the main lumen. The dark area is organizing thrombus, at either end of which is a triangular white zone of variable size and representing fully organized and healed thrombus; the final dissection began at "A" and completed encirclement of the vessel as illustrated by the separation at the ends of the cuts. E. Cut from near the lower openings of the older dissection. These are surrounded by connective tissue.

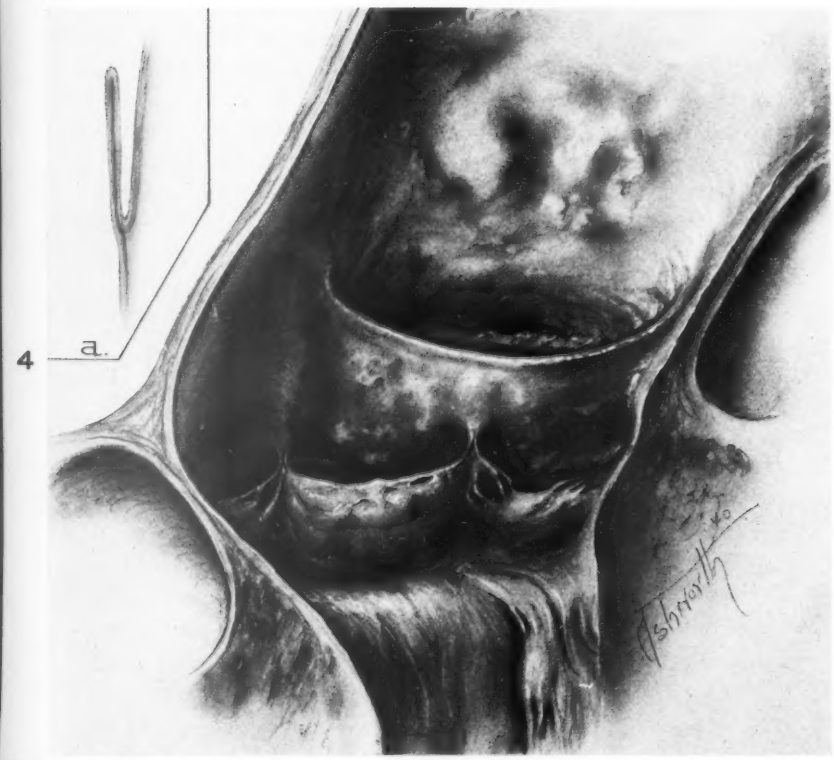
FIG. 4. Case 4 (M-155-6-40). Reduced slightly from a full-size drawing of small and completely healed aneurysm in posterior wall of ascending aorta. There is some thickening and fenestration of the aortic valve cusps. Inserted at *a* is a drawing based on a microscopic section stained for elastic tissue, depicting the extension of fibers (black) into the flap and directly across the base of the defect to connect with elastica in the undissected aortic wall.



A

B





Hunter and Lium Dissecting Aortic Aneurysm

SCOLIOSIS AND DISSECTING ANEURYSM OF THE AORTA IN RATS FED WITH LATHYRUS ODORATUS SEEDS *

IGNACIO V. PONSETI, M.D., and WILLIAM A. BAIRD, M.D.
(*From the Department of Orthopedic Surgery, State University of Iowa, Iowa City, Iowa*)

Epidemic lathyrism has been known to exist for many years in countries where famines have forced the populace to consume a high percentage of legumes in their diet. Humans and other animals with lathyrism have weakness and spasticity of the lower extremities with urinary and fecal incontinence.

Stockman,[1] in 1929, reported his findings of lathyrism in man and also in experimental animals. Geiger, Steenbock, and Parsons,[2] in 1933, noted scoliosis and hernias in white rats fed a diet containing 50 per cent sweet pea (*Lathyrus odoratus*) seed. These authors found that water extracts of the peas fed to rats were toxic while the extracted peas fed at a 50 per cent level were not toxic. Stockman,[3] reporting in 1934, noted that a watery extract of lathyrus peas had a toxic action on the brain and spinal cord. Lewis *et al.*,[4] in 1948, noted spinal curvatures in rats fed various species of legumes. Vivanco and Jiménez Díaz[5] have done extensive studies on the legume intoxication which has been seen in the past few years in Spain. They also found lameness of the hind legs, scoliosis, and periosteal ossifications in the femora of rats fed a diet with large amounts of sweet pea meal. It had been suggested by Lewis that the lameness of the lower extremities was due to compression of the cord at the level of the kyphoscoliosis. Vivanco and Jiménez Díaz performed decompression laminectomies in the rats prior to the appearance of the scoliosis without noticeable effect on the lameness. They postulated that the sweetpea has a toxic action on the spinal cord and bone.

It is well known that legumes are deficient in certain essential amino acids, particularly methionine.[6] However, Lewis[4] proved that the lesions produced by the pea meal are not due solely to a protein deficiency. He observed that the addition of 10 per cent casein did not prevent or delay the onset of lathyrism in rats.

For the past 3 years, metabolic studies of a large group of children with idiopathic scoliosis[7] have been conducted by one of us (I.V.P.) with Dr. Genevieve Stearns of the Department of Pediatrics. It has been found that the protein metabolism is at fault in these children. There are many points of similarity between the roentgenologic characteristics of scoliosis in rats with lathyrism and human idiopathic

* Received for publication, April 28, 1952.

scoliosis. The production of scoliosis in animals by diet afforded an excellent opportunity for a careful histopathologic study of the cause and evolution of this deformity which had not been investigated by previous researchers.

Sixteen 4-week-old white male rats, weighing an average of 68 gm., were divided into three groups containing 8, 4, and 4 animals, respectively. Group I (8 rats) was fed the following diet:

Pea meal (ground *Lathyrus odoratus*)	50%
Cornstarch	28%
Sucrose	6%
Salt mixture	4%
Corn oil	2%
Dried yeast	10%
Halibut liver oil, plain	0.2 cc. per 100 gm.

Group II (4 rats) was fed the above diet with the addition of 10 per cent casein. Group III (4 rats) was fed as group II with the addition of 0.75 per cent methionine. The animals were weighed and roentgenograms were taken under ether anesthesia every week. There were 8 intercurrent deaths and the remaining animals were sacrificed with ether at intervals after the development of scoliosis. Necropsies were performed on all animals.

Three rats of group II were given the stock laboratory diet at 10 weeks of age after they had developed scoliosis. At the same time, casein was added to the diet of 4 rats of group I, and casein and methionine were added to the diet of the other 4 rats of Group I.

Roentgenographic Findings

Roentgenograms taken after 2 to 3 weeks of the pea meal diet revealed generalized demineralization of the skeleton. Shortly thereafter periosteal new bone formation appeared, particularly at the level of the femoral metaphysis. Progressive bowing and deformity of the long bones followed the osteoporosis. During the fourth to sixth week of the diet, narrowing and irregularity of the contour of the intervertebral spaces became apparent at the thoracolumbar junction. This was shortly followed by slight forward slipping of the cephalad vertebra over the caudal vertebra at the level of the intervertebral changes. A week later, there was collapse of two or three vertebral bodies at this level with resulting kyphosis. Lateral slipping and vertebral rotation occurred in from 1 to 2 weeks and a thoracolumbar kyphoscoliosis was then well established (Figs. 1 and 2).

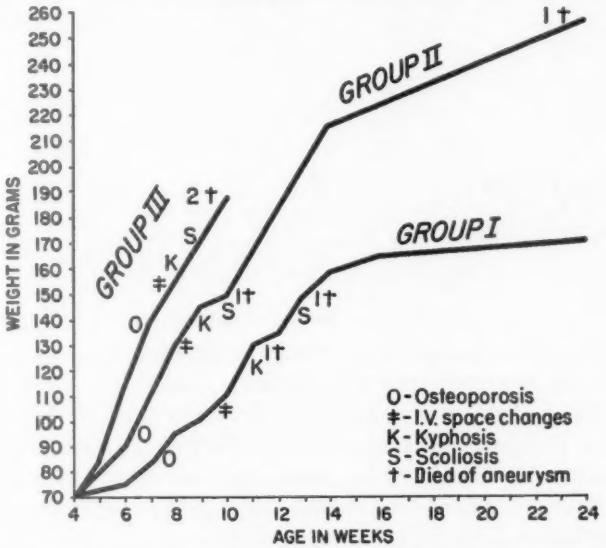
Similar changes occurred in the mid-thoracic area 2 to 3 weeks later, resulting in the formation of thoracic kyphoscoliosis. Costal de-

formity accompanied the rotation of the thoracic vertebrae. All of the rats developed progressive scoliosis and the severity of curvature was related to the duration of the ingestion of the experimental diet. A left thoracolumbar curve was most common. The apex of the thoracic curve was usually at the sixth thoracic vertebra. Well developed lumbar curves were observed in only 2 rats.

The 3 rats of group II which were given the stock diet after they had developed scoliosis manifested a re-ossification of the skeleton in from 2 to 3 weeks. This was followed by straightening of the long bones and disappearance of periosteal new bone formations. The kyphoscoliosis became arrested.

Clinical Course

The addition of casein, and of casein and methionine to the pea meal diet resulted in more rapid growth, as can be seen in Text-figure 1,



Text-figure 1. Comparison of rate of growth and appearance of lesions in the three groups of experimental animals.

and the early growth rate of the animals in group III approached normal. The roentgenographic changes appeared earlier in the animals with more rapid gain in weight. The addition of these two substances in no way altered the development of the kyphoscoliosis and its appearance at a younger age is probably due to the more rapid growth. This finding substantiates the presence of a toxic factor in the pea meal.

Clinically, the animals were unhealthy and did poorly. The kyphoscoliosis and rib cage deformities were obvious. Although they were allowed to eat and drink *ad libitum,* they became thin and listless, with rumpled, sparse, unkempt fur. Two rats developed paraplegia about 10 days before death, with dragging of the hind quarters and incontinence. The remaining rats could climb on the side of the cages but lacked the speed and poise of healthy animals. The animals resumed a healthy appearance when placed on a normal diet and although the kyphoscoliosis persisted, they became fat, sleek, and active.

Necropsy Findings

At necropsy the significant gross findings in the sacrificed animals were confined to the skeleton and the aorta. The thoracic and abdominal organs appeared normal except for underdeveloped testes.

Five rats which died spontaneously 5 to 9 weeks after being put on the pea meal diet had massive bilateral hemothorax. One rat from group II, which was given a normal diet for 15 weeks after development of the scoliosis, died of massive hemopericardium 1 week after resumption of the experimental diet. One death was due to pneumonia late in the experiment. One rat with severe scoliosis and emaciation died in the 19th week without demonstrable specific cause of death.

The 2 rats which had paraplegia at the time of death at 19 and 24 weeks of age demonstrated at necropsy cord compression at the site of the very severe thoracic kyphoscoliosis. Scar tissue was noted over the posterolateral aspect of the cord adherent to the dura mater at this site.

Histopathologic Findings

The first microscopic change noted was osteoporosis of the vertebrae and long bones. The epiphyseal plates appeared normal and endochondral ossification progressed in the usual orderly manner. The cortex of the vertebrae was very thin and the bony trabeculae were thin and widely separated by hematopoietic marrow. No abnormal amount of osteoid tissue was noted. Similar changes were observed in the long bones (Fig. 3).

Extensive areas of subperiosteal new bone formation were noted in the metaphyseal region of the long bones. The thick and cellular periosteum overlay islands of richly vascular young connective tissue which exhibited irregular areas of intramembranous new bone. Rather mature new bone was seen overlying the cortex (Figs. 4 and 5). Similar areas of periosteal new bone formation were observed over the anterior aspect of some of the osteoporotic vertebrae. The new bone trabeculae were oriented perpendicularly to the main axis of the vertebrae (Fig. 6).

No definite early changes of the intervertebral disks could be recognized histologically. The severe osteoporosis led to collapse of the anterior portions of the vertebral bodies. There was an associated forward slipping of the cephalad vertebra which occurred in part at the intervertebral disk and was accentuated by the collapse of the caudal vertebral body. The collapse usually occurred at the anterosuperior portion of the body, just beneath the epiphyseal plate. There was hypertrophy of bone at the site of the collapse suggesting attempted restitution, which did not appear to alter the progress of the deformity (Fig. 7). In the late stages of the scoliosis the involved vertebral bodies showed severe wedging and osteoporosis with no signs of new bone formation. Large masses of fibrous tissue were observed adjacent to and between the vertebrae, which in part replaced the lost contour of the body. The intervertebral disks showed great distortion and narrowing (Fig. 8).

Striking microscopic changes were noted in the aortas of 6 of the 8 animals which died during the experiment. There were irregular, scattered areas of medial necrosis along the thoracic aorta. The necrosis led to weakening of the vessel and the formation of aneurysms. There were extensive areas of "dissection" where the fibers of the media were split by the passage of blood (Fig. 9). Ultimately the blood extended into the adventitia with the formation of large hematomas and rupture into the thorax. One animal revealed a large saccular aneurysm communicating with the lumen of the thoracic aorta by a wide opening (Fig. 11). The media ended abruptly at this point and the intima was not distinguishable from the lining of the aneurysm. The wall of the aneurysm was formed by a thick layer of laminated clot and compressed adventitial tissue. One aneurysm of the arch ruptured into the pericardium. No atheromatous plaques were noted. The other vessels of these animals did not appear unusual.

In 7 animals of varying age, the spinal cord was obtained *in toto* by laminectomy for study with Nissl's, Weil's, and Masson's stains. Histologic study of many levels of the cord revealed no microscopic changes in the cells or tracts except in the 2 rats with paraplegia. At the site of the severe kyphoscoliosis in these 2 animals the posterior funiculi and gray matter were greatly distorted (Fig. 12). The cells of the posterior horns at the site of the compression were largely absent and there was some loss of the anterior horn cells at this level. There was slight gliosis in the corticospinal tract, and local loss of myelin in all funiculi. The cord above and below this level appeared normal. The nerve roots were not unusual.

Histologic sections of the abdominal and thoracic organs, except as

noted, were not unusual. Severe extensive bronchopneumonia was found in one rat. Skeletal muscles did not appear abnormal.

Summary

White rats fed a diet containing 50 per cent *Lathyrus odoratus* seeds all developed kyphoscoliosis. The scoliosis appeared to result from osteoporosis and collapse of several vertebrae. Paraplegia developed in 2 rats with very severe kyphoscoliosis and cord compression.

Aneurysms of the thoracic aorta were seen in 6 of the 8 animals which died intercurrently. The aneurysms developed when the vertebral deformity was still mild.

No toxic lesions were demonstrable in the liver, kidney, or other organs.

Lathyrus odoratus seeds appear to contain a toxic factor which affects the formation of organic bone matrix, the aorta, and possibly other mesodermal structures.

We wish to thank Drs. E. D. Warner and Walter R. Ingram of the Departments of Pathology and Anatomy, State University of Iowa, for help in the microscopic material of these experiments.

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Description of Plates

Plate 163

Fig. 1. Rat IAO. Postero-anterior roentgenograms. (A). Nine weeks of age with generalized osteoporosis and ossification near the upper femoral metaphysis. (B). Eleven weeks of age with bowing and increased periosteal new bone formation in both femora. Early left thoracolumbar scoliosis. (C). Fourteen weeks of age showing advanced osteoporosis and bowing of the long bones. Right thoracic scoliosis with costal deformity. (D). Eighteen weeks of age with increase of deformities noted in C.

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Scoliosis with *Lathyrus odoratus*

PLATE 164

FIG. 2. Rat IAO. Right lateral roentgenograms. (A). Eleven weeks of age with narrowing of the intervertebral spaces at thoracolumbar junction, with forward slipping of T-12 on T-13. (B). Thirteen weeks of age showing advanced slipping of T-12 on T-13 with wedging of T-12 and kyphosis. Early kyphosis at mid-thoracic spine. (C and D). Sixteen and 18 weeks of age, respectively, with advanced kyphosis at mid-thoracic spine. (This rat had compression of the spinal cord and paraplegia.)

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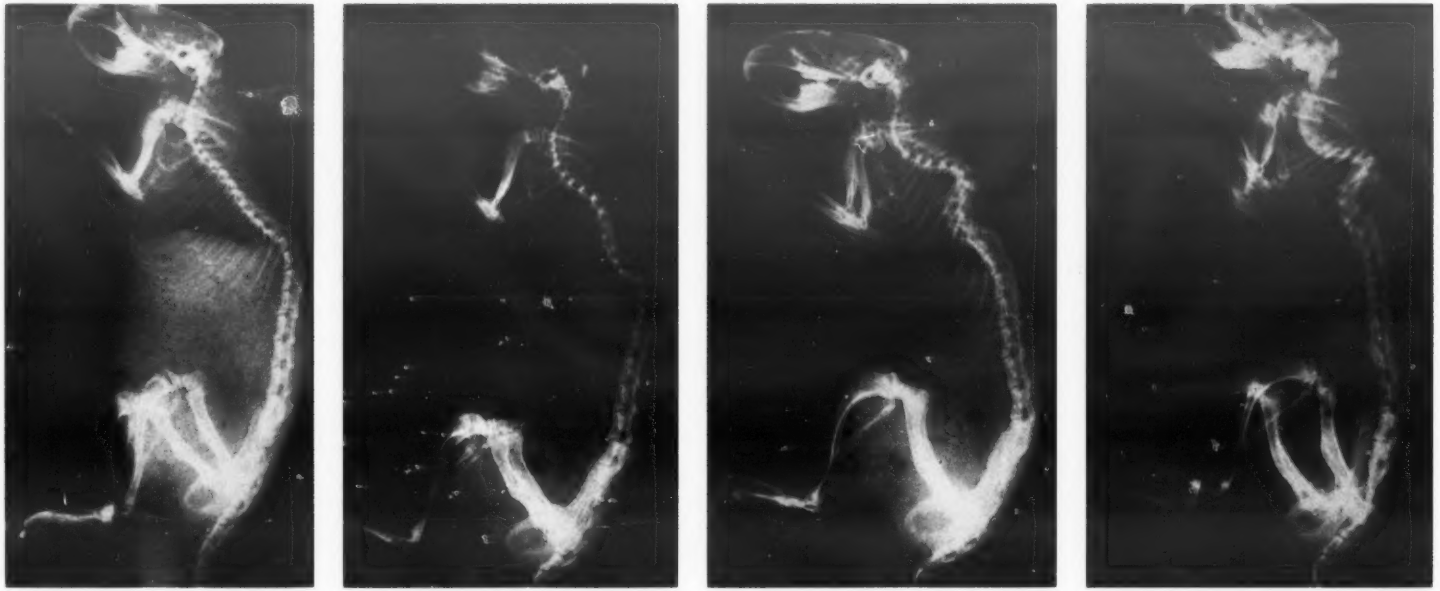

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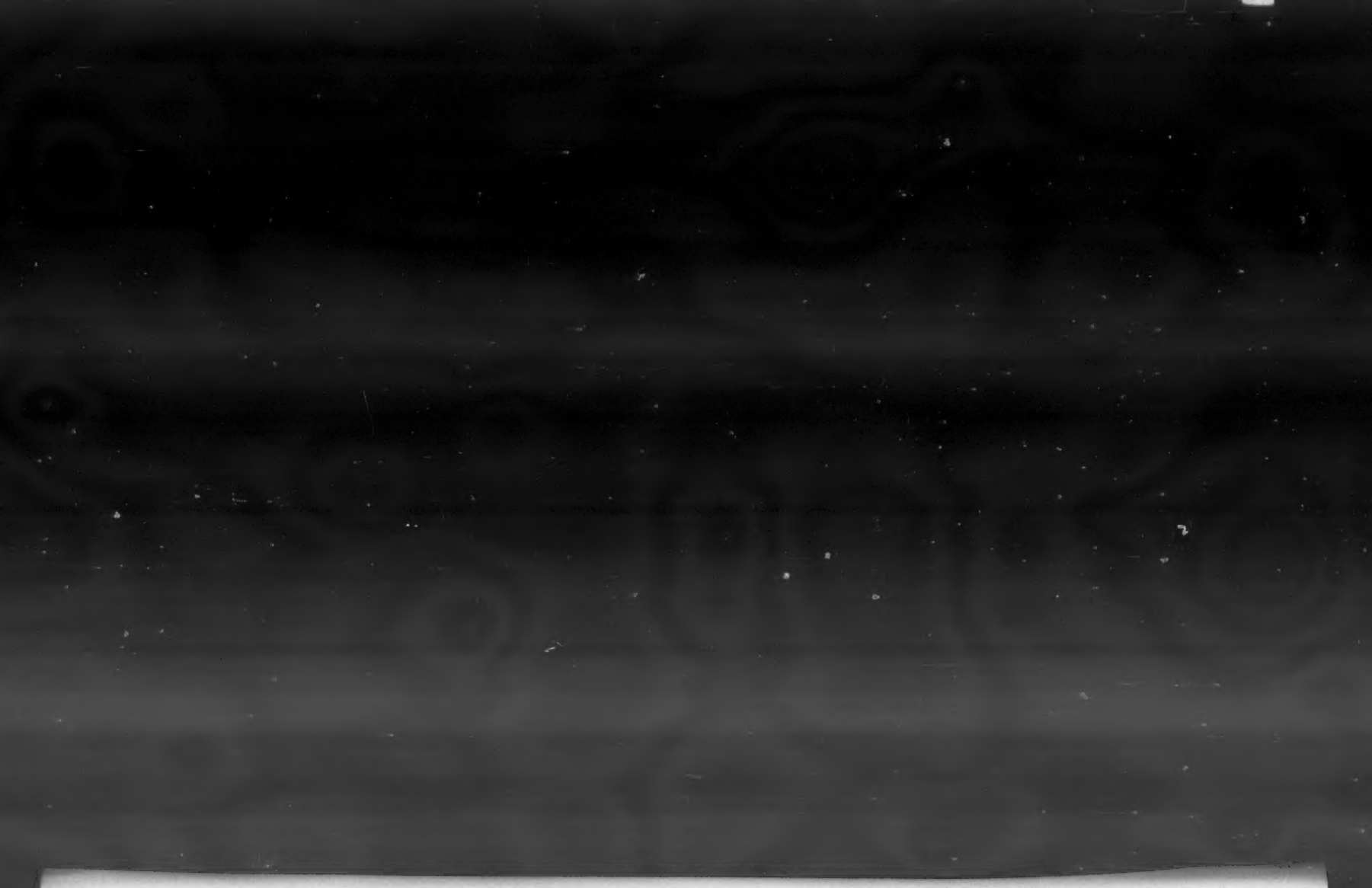
Ponseti and Baird

Scoliosis with *Lathyrus odoratus*

PLATE 165

FIG. 3. Rat IADL (sacrificed at 12 weeks of age). Photomicrograph of vertebra showing osteoporosis and thinning of cortex.

FIG. 4. IBO (died of aneurysm at 11 weeks of age). Photomicrograph of lower femur with periosteal new bone formation and connective tissue beneath periosteum. Endochondral bone formation appears normal.



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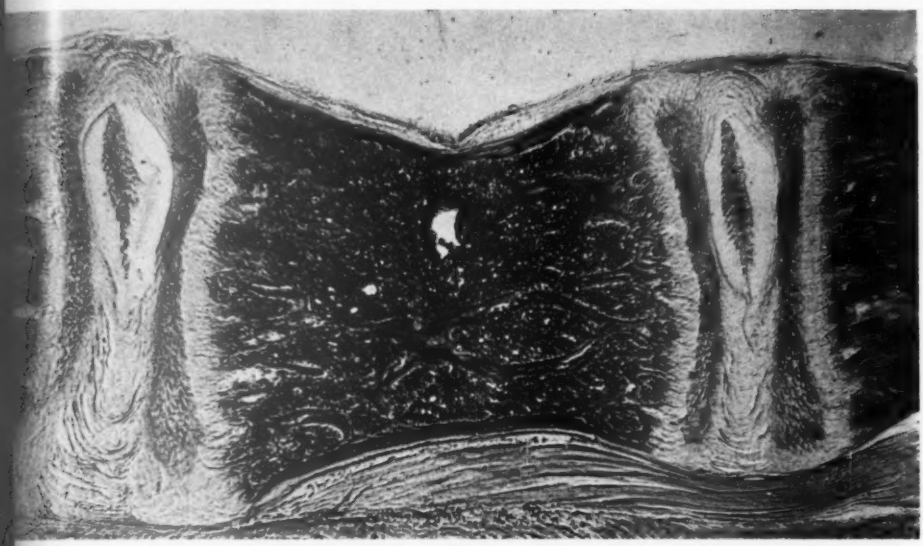

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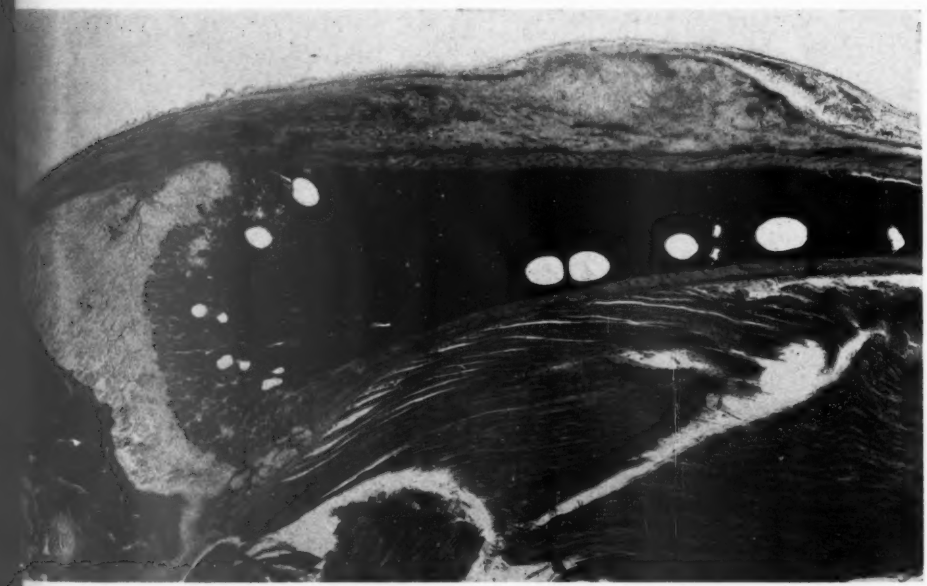

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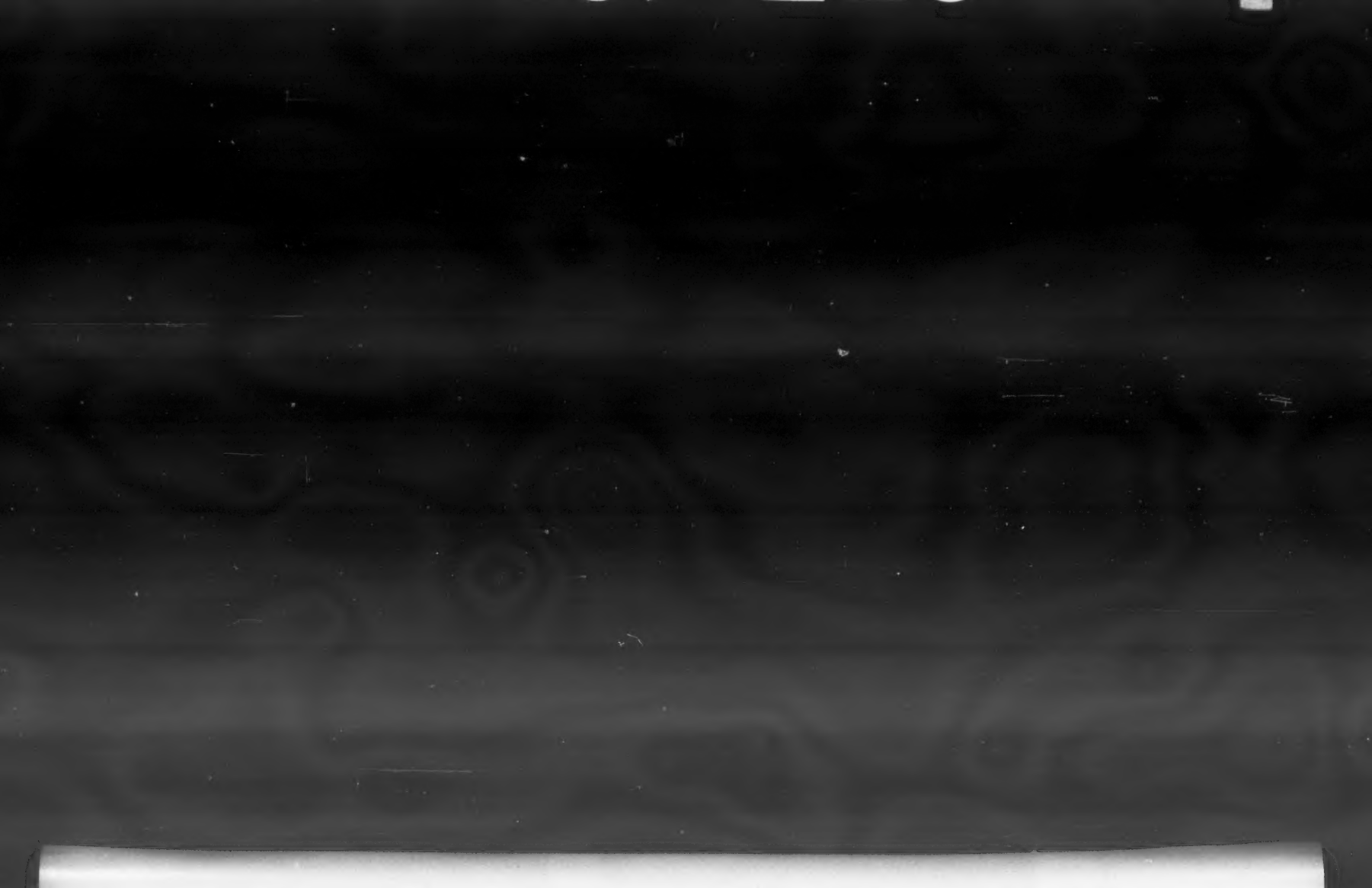
PLATE 166

FIG. 5. Rat IBO (died of aneurysm at 11 weeks of age). Higher magnification of periosteal new bone formation.

FIG. 6. Rat IBDL (died of aneurysm at 13 weeks of age). Photomicrograph of lumbar vertebra showing osteoporosis and periosteal new bone formation over anterior surface of the vertebral body.



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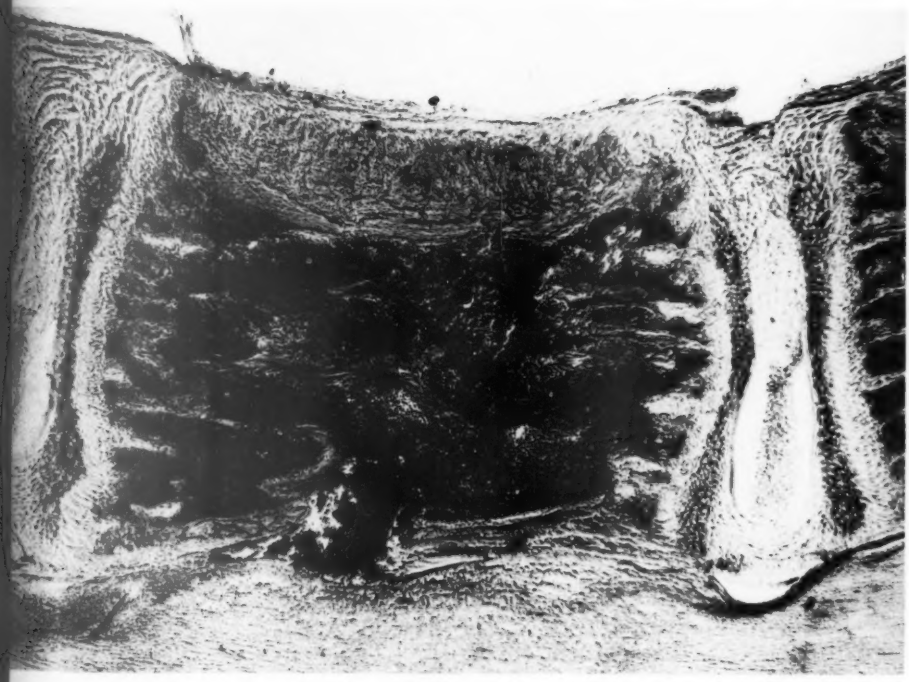

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Ponseti and Baird Scoliosis with *Lathyrus odoratus*

PLATE 167

FIG. 7. Rat IBDL (died of aneurysm of the aorta at 13 weeks of age). Photomicrograph of lower thoracic vertebrae showing collapse of the anterior portion of two vertebral bodies and forward slipping of the cephalad vertebra which is at the right.

FIG. 8. Rat IAL (sacrificed at 24 weeks of age). Photomicrograph of thoracic vertebrae. There is severe wedging and osteoporosis of vertebral bodies with extensive fibrous tissue replacement. The intervertebral disks are thinned and distorted.

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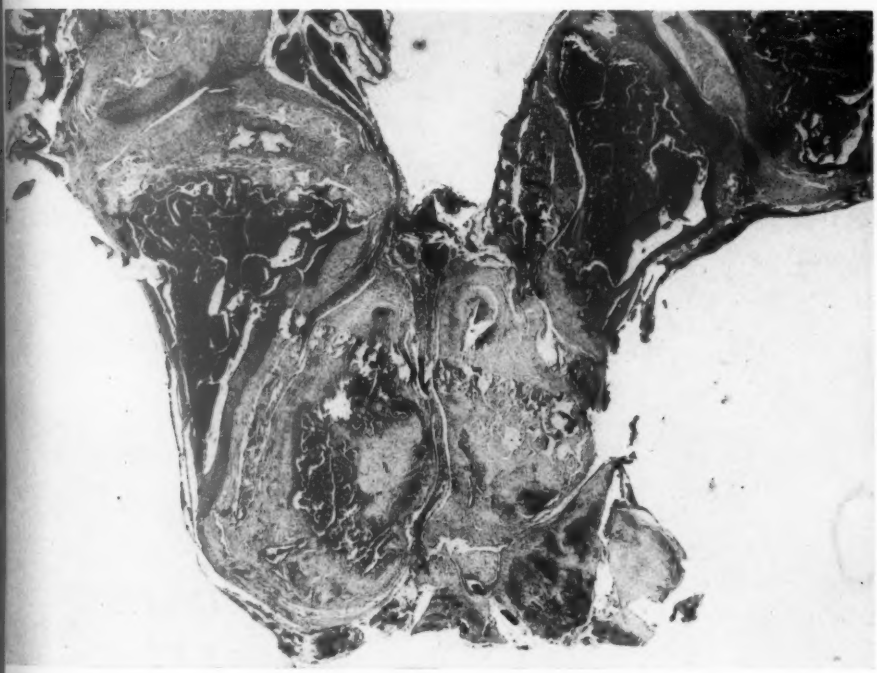

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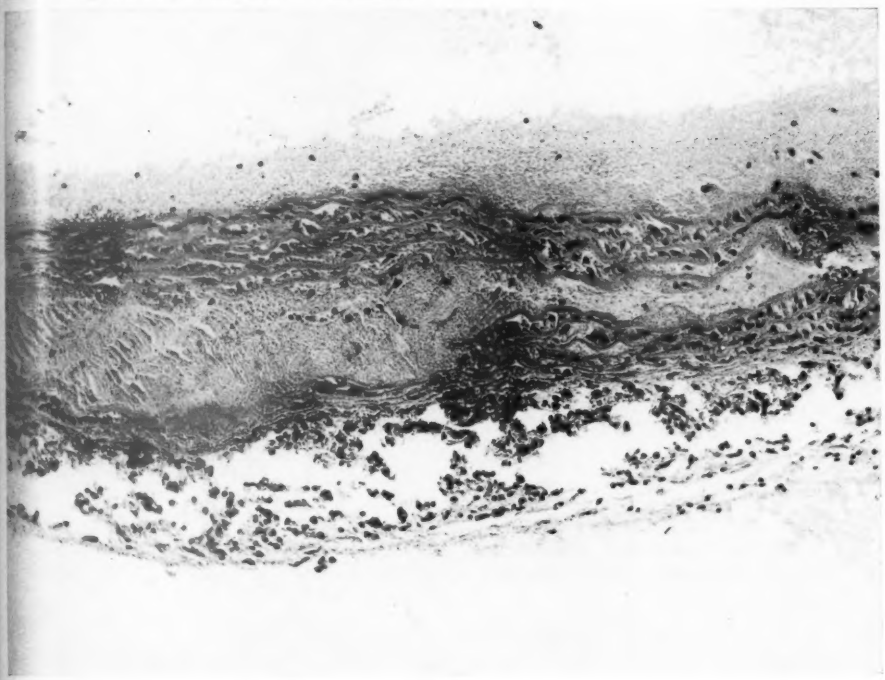
Ponseti and Baird Scoliosis with *Lathyrus odoratus*

PLATE 168

FIG. 9. Rat IBO (died of aneurysm at 11 weeks of age; had minimal kyphoscoliosis). Photomicrograph of the wall of the thoracic aorta showing medial necrosis and separation of the fibers of the media by blood.

FIG. 10. Rat IBO (died of aneurysm at 11 weeks of age; had minimal kyphoscoliosis). High-power photomicrograph of the wall of the aorta.



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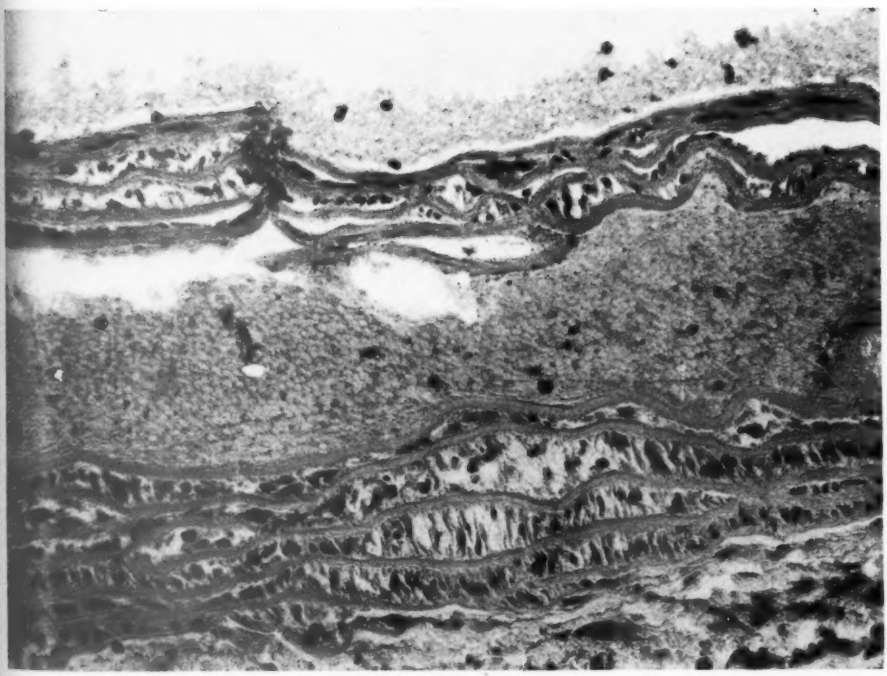

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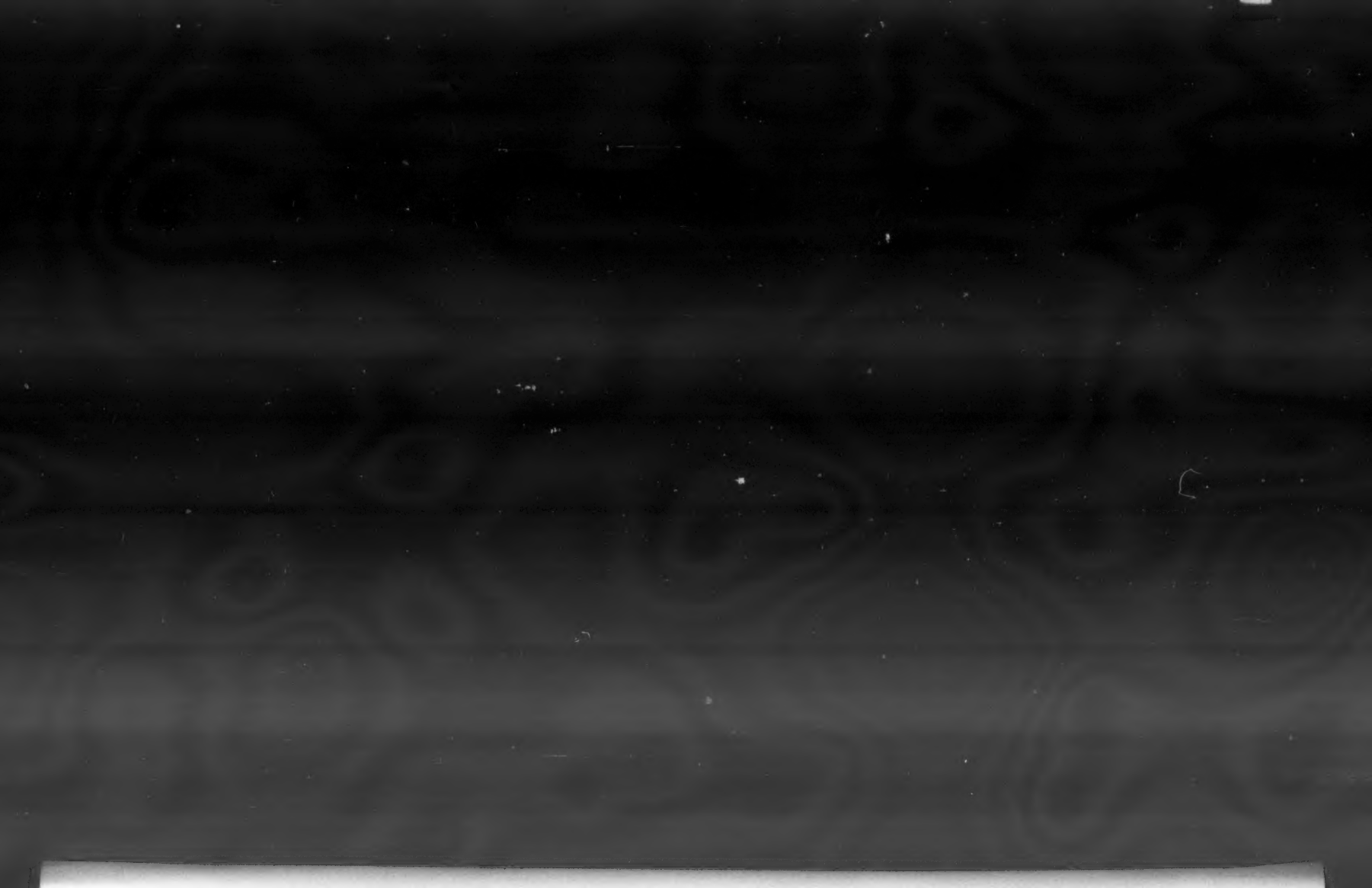
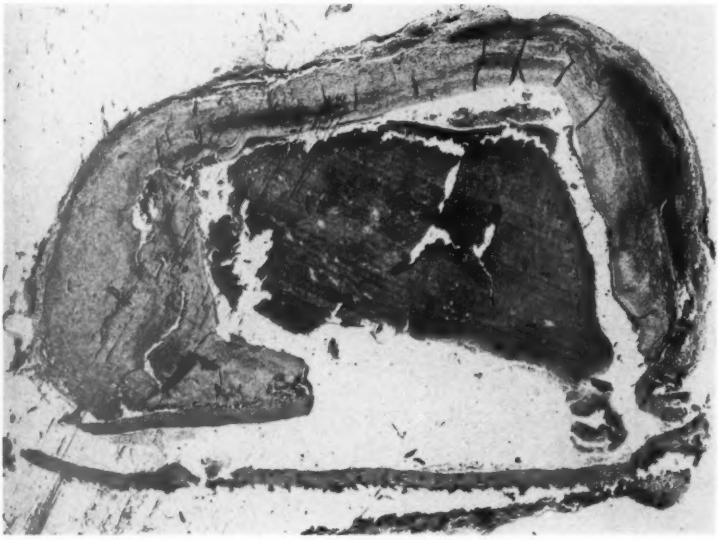
Ponseti and Baird Scoliosis with *Lathyrus odoratus*

PLATE 169

FIG. 11. Rat IBDL (died of aneurysm at 13 weeks of age). Photomicrograph of a large saccular aneurysm in the thoracic aorta.

FIG. 12. Rat IAL (sacrificed at 24 weeks of age). Photomicrograph of spinal cord at level of severe kyphoscoliosis, Nissl's stain. There is compression of the posterolateral aspect of the cord.





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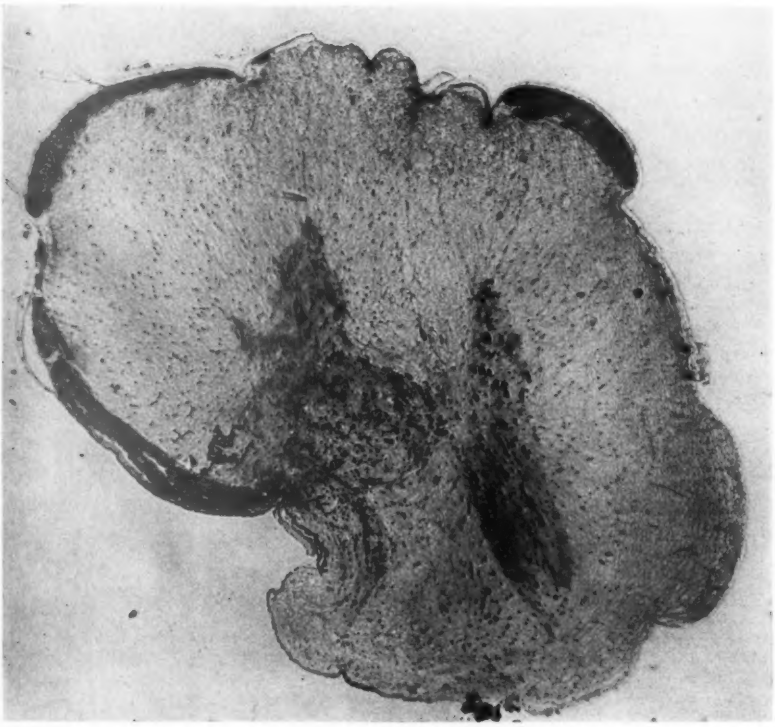

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Ponseti and Baird Scoliosis with *Lathyrus odoratus*

PATHOLOGIC CHANGES IN THE GASTRIC MUCOSA OF THE RAT AFTER HISTAMINE ADMINISTRATION *

PERIHAN CAMBEL, M.D., and JAMES T. SCOURIS, M.S.

(From the Cancer Research Laboratory, University of Florida, Gainesville, Fla.)

Bowie and Vineberg[1] showed that while repeated vagal stimulation depleted the dog's peptic cells of their zymogen content, no such effect was obtained by hourly histamine injections over a period of 8 hours. Recently, Dalton[2] found that C_3H mice receiving 0.5 mg. of histamine hydrochloride per gram of body weight intraperitoneally and killed 3 hours later or after fasting 24 hours, remained unaffected according to electron microscopic studies.

No histologic investigation on the rat's peptic, parietal, and mucous cells during histamine administration was found in the literature. Therefore, a histologic and histochemical study during prolonged histamine treatment seemed of interest despite the reported resistance of rats to single histamine injections[3,4] in doses effective in stimulating gastric secretion in the dog.

EXPERIMENTAL PROCEDURES AND METHODS

Forty albino rats of the Sprague-Dawley-Holtzman strain of both sexes were used. The age of the animals at the onset of the experiment was about 40 days. Their average weight was 200 gm., ranging from 165 to 240 gm. They were fed purina dog chow and supplied water *ad libitum*.†

An aqueous histamine dihydrochloride solution in a concentration of 0.040 mg. per ml.[1] was stored at 17° C. but allowed to stand at 25° C. for 3 hours prior to administration. Subcutaneous injections of 1 ml. were made daily into the abdominal region of each treated rat. The site of injection was alternated from the right to the left side of the abdomen. The rats were starved 36 hours and deprived of water 3 hours prior to sacrifice by neck-stroke. The last injection was administered 2 hours before death.

At necropsy, three smears of the gastric secretion were made. The stomach was cut according to our standard procedure[5] for histologic study.

Each of the four groups of 10 rats comprised 5 females and 5 males. Group I consisted of the non-treated controls; group II was injected

* This investigation was supported by Research Grant C-976 from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

Received for publication, June 13, 1952.

† The local water supply is fluorinated one part to a million.

daily for 1 week; group III, for 2 weeks; and group IV, for 4 weeks (Table I).

The smears of the gastric secretion were stained as described previously.[6] The stomachs were fixed and stained according to our modification of Bowie's method.[5] Stomach sections were stained also with hematoxylin and eosin and with van Gieson's connective tissue stain.

TABLE I

Total Doses of Histamine Dihydrochloride

Group	No. of animals	Duration of treatment	Days of treatment	Histamine
				mg.
I	10	Non-treated controls	0	0.00
II	10	1 week	7	0.28
III	10	2 weeks	14	0.56
IV	10	4 weeks	28	1.12

RESULTS

Gross Findings. The weights of the rats showed variations in the different groups at necropsy (Table II). While in group I a slight decrease in weight was noticed, and the animals looked thinner, those of groups III and IV went up slowly during the first week, and later.

TABLE II

Weights of the Rats at Necropsy

Group	Gm.
I	180–225
II	175–220
III	170–225
IV	210–250

In all experimental groups, decrease in size of the spleen, hyperplasia of Peyer's patches and of lymph nodes of the aortal bifurcation, and atrophic thinning of the stomach wall were noted. The pancreas also showed atrophy and reduced consistency. These changes were most pronounced in group IV.

Smears of Gastric Secretions. The results from smears of gastric secretions have been reported elsewhere.[6,7]

HISTOLOGIC FINDINGS

Group I (Controls)

Zone I. Hematoxylin and Eosin Sections. The squamous epithelium of the esophagus and of the valvular portion of the esophageal orifice varied in thickness and in the number of its layers. The stratum granulosum was invariably present, but varied in thickness and in the amount of keratohyaline granules. These ranged from fine to coarse granules and were sometimes clumped. In some cases slight epithelial

hypertrophy and acanthosis were noted. Neutrophilic polymorphonuclear leukocytes were observed lying in the submucosa.

The valvular portion of the esophageal orifice ended abruptly to give place to the transitional glandular epithelium from which it was separated by a deepening of the mucosa (Fig. 4). The same deepening was seen also adjacent to the "ridge." This is a squamous epithelial fold separating the two main portions of the rat's stomach, the forestomach which is lined with squamous cell epithelium similar to that of the esophagus and the glandular stomach, with the mucosal deepening between squamous cell epithelium and transitional epithelium occasionally containing mucus and desquamated columnar cells.

The transitional epithelium adjacent to the squamous cell epithelium was composed of only a few cells. It increased gradually in thickness toward the compound mucosa (Fig. 4). Near the ridge or valvular portion of the esophageal orifice it was made up mainly of columnar mucous cells. Among these were noted occasional cells resembling the intestinal goblet cells and small spherical, light-blue-staining cells. Only rarely could parietal cells be seen in the portion of the transitional epithelium adjacent to the glandular mucosa.

The mucosa of the anterior and posterior wall of the glandular stomach was usually, but not continuously, covered with a thin coat of mucus, staining light pink. Mucus was observed occasionally in the upper portions of the glandular lumina. The mucous cells, which made up the surface epithelium in the anterior and posterior walls and the larger curvature, were columnar with pink-staining cytoplasm. Their nuclei stained light blue, while the nucleoli were dark blue. In the two walls the lower third of the glands was made up of peptic cells with parietal cells lying between them. In the median portion of the mucosa they dominated the picture, forming cellular strands. Single parietal cells could be seen between the superficial layers of mucous cells.

In the greater curvature was found the thickest wall of the glandular stomach. Here the peptic cells took up two-thirds of the mucosa and the parietal cells one-third. As in the walls, the oxyntic cells were wedged between the pepsinogen cells, and showed the same distribution. Neutrophilic leukocytes usually were seen in the submucosa and to a lesser extent in the interstitial connective tissue between the glands of the mucosa.

Biebrich Scarlet-Ethyl Violet and Hematoxylin Sections. In the transitional epithelium an occasional small parietal cell staining scarlet and a few cells staining diffusely violet blue were observed.[1] Here, no or occasional (Fig. 4) cells with pepsinogen granules were seen. The parietal cells in other regions were stained light scarlet and stood out

in sharp contrast with the dark blue peptic cells. Among the former, binucleation was observed occasionally. The oxyntic cells nearest the mucosal surface did not completely fill their spaces. Intracellular canaliculi were noted only in a few of the parietal cells. Some of these canaliculi seemed to be continuous with those of adjoining parietal cells and with the pericellular spaces. Others opened into the glandular lumina. The lower two-thirds of the mucosa was made up of peptic cells, packed densely with violet-blue-staining pepsinogen granules (Figs. 1 and 7). The latter masked the nuclei. The violet granules were less sharply outlined near the free borders of the peptic cells. Occasionally a scarlet parietal cell was found wedged between them. In a few of these oxyntic cells two to five violet-blue granules were present.

The mucosa of the greater curvature showed the same composition as that of the anterior and posterior stomach walls. However, in the larger curvature the lower two-thirds of the mucosa was made up of peptic cells packed densely with pepsinogen granules. Here the peptic cells were present in largest numbers (Fig. 6).

Zone II. Hematoxylin and Eosin Sections. The lesser curvature was composed mainly of mucous cells. The cellular topography of the gastric walls and the larger curvature was practically identical with that of the same regions in zone I.

Biebrich Scarlet-Ethyl Violet and Hematoxylin Sections. The lesser curvature was void of cells containing violet blue granules. However, a few diffusely blue-staining cells were seen. Although an occasional scarlet parietal cell could be noted, the main components of this region were the mucous cells. The gastric walls and the larger curvature resembled in their cellular distribution the same regions in zone I.

Zone III. Hematoxylin and Eosin Sections. The different regions corresponded in their cellular distribution to those of zone II.

Biebrich Scarlet-Ethyl Violet and Hematoxylin Sections. The composition of the lesser curvature and the gastric walls was identical with the corresponding regions in zone II. However, a greater number of peptic cells were seen in the greater curvature of this zone as compared to zones I and II. These cells were packed even more densely with dark blue pepsinogen granules which masked the nuclei and gave the cells a dark blue color. The density of the granules, therefore, was markedly greater than in zones I and II.

Group II (1 Week Histamine-Treated Animals)

Zone I. Hematoxylin and Eosin Sections. The cells of the transitional epithelium varied in staining properties. Those nearest the mus-

cularis mucosae were basophilic, while those of the surface of the mucosa were eosinophilic. Cells resembling goblet cells and filled with non-staining mucus seemed to be present in slightly increased number as compared with the controls. The surface mucus stained darker pink than in the controls. Here, also, only a few parietal cells were noted. In the walls a decrease in peptic cells and an increase in parietal cells were observed. In the depths of the folds, however, no such changes could be noted. The parietal cells filled out their spaces, were increased in size, and had a bloated appearance. This was accompanied by increased eosinophilia. The greater curvature had no mucous coat. The parietal cells were swollen and markedly eosinophilic. No change in the peptic cells could be detected.

Biebrich Scarlet-Ethyl Violet and Hematoxylin Sections. In the transitional epithelium a few cells staining diffusely blue were observed. In the walls parietal cells were increased while the peptic cells in the lower third of the epithelium showed a decrease in number and in the density of their granules. The distribution of the cells was not changed in the depths of the folds. In the greater curvature the peptic cells showed a decrease in number and in density of granules. The rows of scarlet parietal cells made up all of the median portion and parts of the lower portion of the mucosa. These cells were markedly increased in size. Only a few rows of violet-blue peptic cells contrasted sharply with the increased oxyntic cell strands.

Zone II. Hematoxylin and Eosin Sections. In the lesser curvature the mucous cells seemed to have lost their cohesiveness. Less mucous secretion was present. Here, also, the mucus stained a deeper pink. It contained cellular débris, many free nuclei, and lined the mucosal surface only intermittently. The cell nuclei were less distinct and no peptic or parietal cells were present. The gastric walls showed a decrease in mucous cells, with an increased number of parietal cells throughout the epithelium. They had replaced the correspondingly decreased peptic cells in the lower third of the mucosa.

Biebrich Scarlet-Ethyl Violet and Hematoxylin Sections. No peptic cells were seen in the lesser curvature. The gastric walls and the greater curvature showed decreased pepsinogen granules and peptic cells.

Zone III. Hematoxylin and Eosin Sections. The histologic pictures of the lesser curvature and gastric walls were identical with those of the corresponding regions in zone II. The greater curvature, however, showed that the parietal cells had increased to dominate the upper half of the mucosa. With equal numbers of peptic cells they made up the lower half of the mucosa.

Biebrich Scarlet-Ethyl Violet and Hematoxylin Sections. The histo-

logic pictures of the lesser curvature and gastric walls were identical with those of the corresponding regions in zone II. In the greater curvature the peptic cell distribution was limited to the lower half of the mucosa. Here, they were partially replaced by parietal cells. The peptic cells were lighter violet due to the decrease in pepsinogen granules. Increased leukocytes were observed in the interstitial connective tissue between the glands near the mucosal surface. The mucous cells in this region had lost their cohesiveness. Desquamated cells were noted.

Group III (2-Weeks Histamine-Treated Rats)

Zone I. Hematoxylin and Eosin Sections. No mucus was noted on the mucosal surface of the lesser curvature. Shed cells were increased as compared with the control rats. The glandular lumina were enlarged and lined by increased cuboidal mucous cells, which seemed to replace the shed columnar cells in the mucosa. Leukocytes were increased markedly in the interstitial connective tissue near the mucosal surface. The larger curvature and both gastric walls showed almost the same histologic picture. Many desquamated cells, free pyknotic nuclei, and nuclear contours in the cellular débris on the mucosal surface were indications of cellular destruction and shedding. Very little surface mucus was observed. The mucous cell layer in the anterior and posterior walls was decreased in thickness. Only few mucous cells were seen adjacent to the muscularis mucosae. Many parietal cells were seen near and at the mucosal surface. However, they were small (5 μ) and did not fill their spaces, while those in the lower mucosal portions were large and bloated (15 to 20 μ). At first sight, the parietal cells seemed markedly increased in all portions of the mucosa. This apparent increase, however, seemed partly due to their bloated state. Thus, in the lower third of the mucosa, they took up equal space with the peptic cells, which were actually present in large numbers (Fig. 2). An increase in leukocytes in the interstitial connective tissue was seen in the upper half of the epithelium.

Biebrich Scarlet-Ethyl Violet and Hematoxylin Sections. Although the lesser curvature contained no peptic cells with pepsinogen granules, cells with a diffuse blue color were present. The walls of the stomach showed a decrease in peptic cells and in their pepsinogen granules. The nuclei were more evident because the fewer pepsinogen granules were not able to mask them as in the other sections. Bloated parietal cells partly replaced the peptic cells. In the upper and mid-portions of the mucosa the parietal cells dominated the picture. Both cell types were represented in the lower mucosal portion. The parietal cells appeared to press upon the peptic cells because of their bloating.

Thus, the latter appeared in thin strands. The peptic cells of the larger curvature, which were normally densely distributed in the lower two-thirds of the mucosa, made up only one-half of the epithelium. Pepsinogen granules were markedly decreased so that some nuclei were not masked by them. Here also, parietal cells were replacing peptic cells. Binucleation of parietal cells and some mitotic figures were seen here, but they were found in equal numbers in the controls.

Zone II. Hematoxylin and Eosin Sections. The lesser curvature showed a thin, non-continuous, dark pink mucous coat. Marked desquamation was seen in the lateral areas, with little or no mucus present. Parietal cells were present in large numbers under and between the few surface mucous cells. The lower half of the mucosa was made up of parietal and peptic cells showing the same characteristics as in zone I. However, a new feature was that the glandular lumina were slightly enlarged. Due to the great amount of desquamation, the greater curvature showed a decrease in mucous cells. No mucous layer was covering the surface here. Again the parietal cells had replaced the decreased peptic cells in the lower half of the mucosa.

Zone III. Hematoxylin and Eosin Sections. The lesser curvature showed a marked decrease in the surface mucus. The mucous cells lining the lumina showed vacuoles of unstained mucus. They resembled goblet cells. The gastric walls and the greater curvature showed the same histologic features as in zone II.

Group IV (4-Weeks Histamine-Treated Rats)

Zone I. Hematoxylin and Eosin Sections. The same changes in the cellular distribution were observed as described in the 2-weeks-histamine treated rats. However, they were more marked. Furthermore, mucous cells in the lesser curvature contained non-staining mucous vacuoles so these cells simulated goblet cells (Fig. 4). In group III this change was observed only in zone III. The changes in the gastric walls and the larger curvature were more marked than in zone III of group III, because the peptic cells now made up only one-half of the lower half of the mucosa. No surface mucus was present in any region. An increased number of neutrophilic leukocytes was discernible in the interstitial connective tissue.

Biebrich Scarlet-Ethyl Violet and Hematoxylin Sections. The changes were the same as those observed in the hematoxylin and eosin sections. The contrasting quality of the stain made them stand out more markedly. In the gastric walls and the greater curvature the zymogen granules in the peptic cells had decreased to such an extent that the cells appeared light violet (Fig. 3). In the greater curvature

the decrease in number of the peptic cells was the most marked (Figs. 3 and 5).

Zone II. Sections stained with hematoxylin and eosin and the Biebrich scarlet-ethyl violet stain showed the same changes as those observed in zone I. The goblet-like cells lining the surface of the lesser curvature were in greater numbers than had been observed before. The glandular lumina had enlarged.

Zone III. The histologic picture of the lesser curvature was identical with that of zone II with both stains. The peptic cells present in the lower portion of the mucosa were even less abundant. In sections stained by van Gieson's method no special features were noted.

Discussion

The Effect of Histamine on Parietal Cells and Hydrochloric Acid Secretion

A progressive thickening of the gastric mucosa due to an increase in the size and number of the parietal cells, some of which contained a few pepsinogen granules, was observed in histamine-treated rats. The intracellular canaliculi stood out conspicuously. In the 4-weeks histamine-treated rats enlarged parietal cells often replaced peptic cells, which were much decreased in number and were also compressed by them. These histologic findings indicated a stimulating effect of histamine on the parietal cells, comparable to that observed by Cox and Barnes[8] in the guinea-pig. These authors believed that the increase in the parietal cells represented a response to stimulation of function. They concluded that this response was a true experimental hyperplasia of the gastric mucosa. They failed to recognize partially granulated peptic cells. This, probably, was due to the use of the Giemsa stain instead of a histochemical method which would demonstrate zymogen granules.[5] However, several authors[3,4] have maintained the concept of histamine resistance in the rat. While searching the literature, an article by Voss[9] attracted our attention. This article, which appeared in 1938, has been largely overlooked. It gave experimental proof that the so-called histamine resistance of the rat is only relative. One of the reasons for the discrepancy between Voss's results and ours, and the literature cited above,[3,4] may lie in the fact that we, like Voss, did not anesthetize our rats and so avoided a possible inhibitory effect on the histamine stimulation of gastric secretion. Another reason for the discrepancy may be the difference in the modes of administration. While Voss[9] and we used repeated administration over prolonged periods, the other workers used either single injections[3,4] or a short period of administration.[1]

A sex difference in the response of the rat's parietal cells to histamine was not observed, in harmony with the results of Cox and Barnes[8] in the guinea-pig.

It is concluded from our experimental results that the unanesthetized albino rat can respond to repeated, prolonged histamine injections with parietal cell hyperplasia. This is accompanied by widening of the intracellular canaliculi which may be morphologic evidence of stimulated acid secretion.

The Histamine Effect on Pepsinogen Cells and Pepsin Secretion

The peptic cells were gradually depleted of zymogen granules. They also decreased in number and size. The latter was due to the compression exerted upon them by parietal cells which had increased in number and size. No sex difference in this response of the peptic cells was noted.

No histochemical alterations of the peptic cells were observed by Bowie and Vineberg[1] in their histamine-treated dogs. The difference between our results and theirs may be due not only to the different modes of administration, but also to biochemical difference between the two species. Popielski[10] established a species difference in regard to the histamine response in the human and the dog, the human being less sensitive than the canine. Northrop's[11] finding of a different molecular configuration in the protein structure of pepsin of different species lends support to this view. Therefore, it may be postulated that histamine may affect differently pepsin molecules of different structure.

Bucher, Ivy, and Gray[12] and later Bucher[13] observed that histamine stimulated the peptic cells and increased pepsin secretion in dogs with vagotomized entire stomach pouches. Toby[14] found that histamine caused an abundant secretion of pepsin in the human stomach. On the other hand Alley,[15] working with Pavlov pouched dogs, showed that histamine had an inhibitory action on the peptic cells.

The findings of Gilman and Cowgill[16] in pouched dogs seem to explain the discrepancy between the results of Toby[14] and Alley.[15] According to Gilman and Cowgill,[16] an initial rise in the pepsin of the gastric juice followed histamine administration. After this transitory stimulation the total pepsin fell rapidly. We assume that the decrease of the zymogen granules in the peptic cells of the rat may be attributed to an inhibitory action on zymogen production after increased pepsin output. The pressure exerted by the bloated parietal cells on the peptic cells may, on the other hand, have caused a mechanical inhibition of the zymogen production. Correlated morphologic and physiologic studies are necessary to throw more light on these mechanisms.

The Histamine Effect on the Mucous Cells and the Mucous Secretion

In the 1-week histamine-treated rats a dark-staining, thick surface mucus was observed. In the experimental groups treated longer than 1 week, surface mucus was either decreased or absent. The intercellular cement of the mucous cells was loosened. Pronounced exfoliation of columnar cells was observed as evidenced by the presence of columnar cells among the cellular débris on the mucosal surface. Large areas, particularly in the larger and lesser curvatures, showed dark-staining regenerated columnar and cuboidal cells partly replacing the exfoliating mucous cells. Most of these, in the 4-weeks treated rats, contained non-staining mucous vacuoles. Thereby, they resembled the intestinal goblet cells. These goblet-like cells may be compared with the intestinalization of the gastric mucosa described by Howes.[17] This author advanced the hypothesis "that whenever intestinalized epithelium is found in areas where combined epithelium (acid, pepsin, and mucous-secreting cells) ordinarily exists, injury must have occurred." Cox[18] considered "metaplasia of the epithelium to form intestinal-type glands" as one of the criteria in the identification of chronic gastritis. He graded the process as mild, moderate, or severe. Pointing out that chronic atrophic gastritis is not a single morbid entity, Cox stated that "the possible relationship [of chronic atrophic gastritis to gastric carcinoma] should be investigated further, giving attention to possible differences in the type of gastritis which is found in association with gastric carcinoma."

The occasional presence of goblet-like cells in the transitional epithelium and in the simple mucosa of the lesser curvature of the control rats was observed in this and another investigation.[19] The natural occurrence of such cells and their increase in the same regions under histamine treatment can be considered a manifestation of the inherent, often latent potency of the gastric mucosa to form epithelium resembling the intestinal type. Partial intestinalization of the simple epithelium in the lesser curvature and of the combined epithelium in the larger curvature can be interpreted as metaplastic regeneration in histamine injured areas. This change was accompanied by increase in number and size of the parietal cells and a depletion of the zymogen granules. It is concluded that the alterations found in the rat's gastric mucosa after histamine treatment can be designated as regenerative, hypertrophic, metaplastic gastritis accompanied by zymogen depletion.

No sex difference was noted.

Summary

Prolonged administration of histamine dihydrochloride increased the width of the compound gastric mucosa of the unanesthetized albino rat. This was due to increase in number and size of the parietal cells. Simultaneously, a gradual decrease in surface mucus, exfoliation of the mucous cells, and a gradual depletion of the zymogen content of the peptic cells were observed. The depletion increased with the length of treatment. A partial intestinalization was observed in the 4-weeks histamine-treated rats.

It is concluded, therefore, that repeated and prolonged histamine administration causes parietal cell hyperplasia, depletion of the zymogen content, and finally partial intestinalization. These findings are experimental and provide histologic evidence favoring our opinion that, contrary to maintained views, the rat is only relatively resistant to histamine. The alterations produced by histamine in the mucosa can be designated as a hypertrophic, metaplastic gastritis accompanied by regeneration, decrease of surface mucus, and zymogen depletion.

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DESCRIPTION OF PLATES

PLATE 170

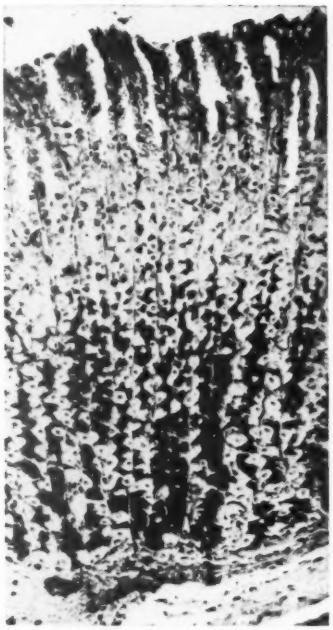
Gastric mucosa showing gradual depletion of pepsinogen granules. All figures prepared following modified Bowie's staining method. × 125.

FIG. 1. In non-treated rat.

FIG. 2. After 2 weeks of treatment.

FIG. 3. After 4 weeks of treatment.

1



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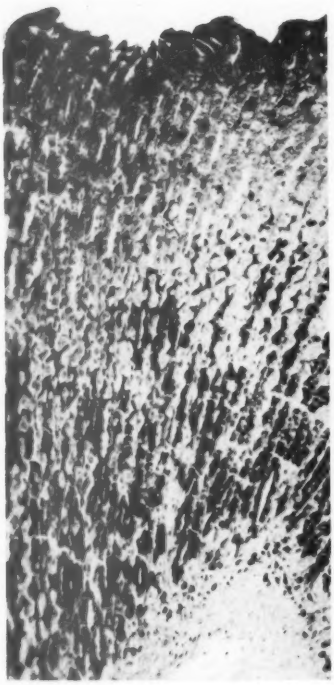
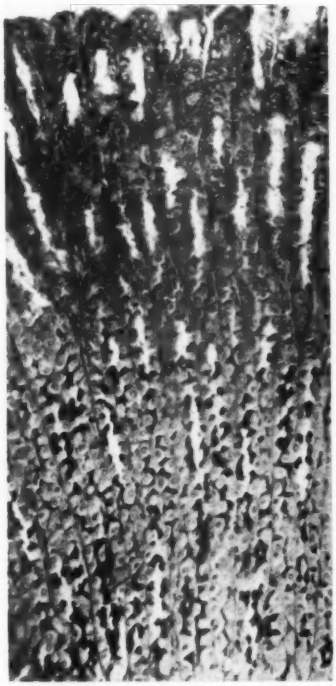




3

Cambel and Sgouris Gastric Mucosa of Rat after Histamine

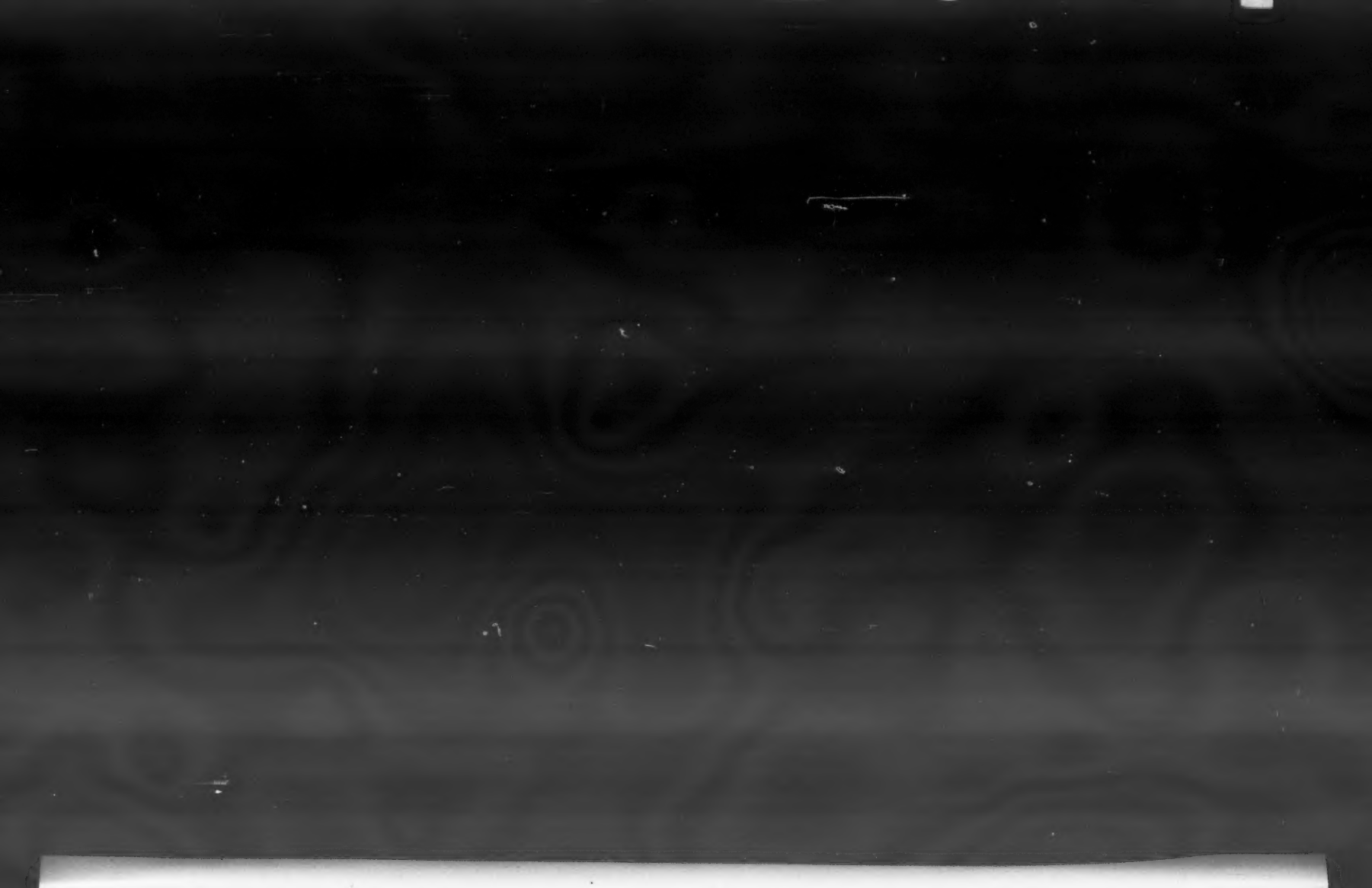
PLATE 171

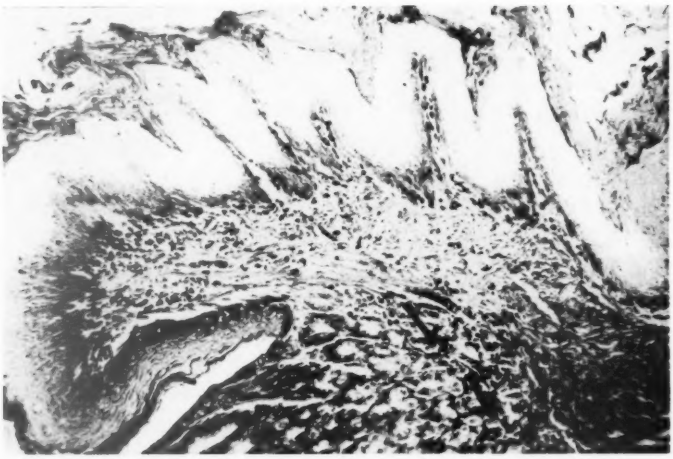
FIG. 4. Transitional epithelium with occasional pepsinogen cells indicated by arrows (non-treated rat). × 125.

FIG. 5. Transitional epithelium devoid of pepsinogen cells (4 weeks histamine-treated rat). × 125.

FIG. 6. Pepsinogen cells near larger curvature stained with modified Bowie's method. × 125.

FIG. 7. Simple mucosa of small curvature showing dominance of goblet-like cells in 4 weeks histamine-treated rat (intestinalization). × 125.





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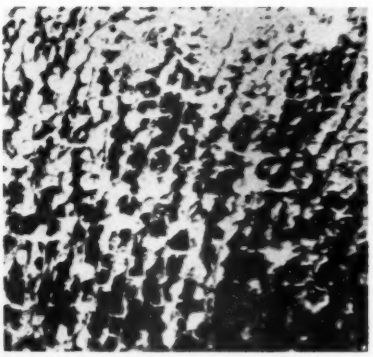

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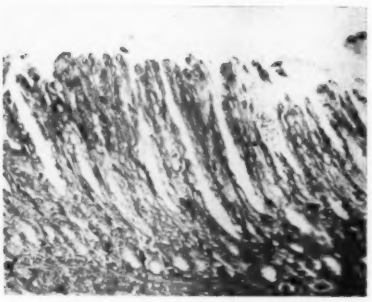

7

Cambel and Sgouris

Gastric Mucosa of Rat after Histamine

GIANT CELL MYOCARDITIS *

B. H. KEAN, M.D.,† and MARK T. HOEKENGA, M.D.‡
(*From the Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone*)

The first description of granulomatous, giant cell myocarditis, apparently unassociated with tuberculosis, syphilis, or other demonstrable cause, was made in 1905 by Saltykow.[1] Since then, additional reports of this lesion have been published infrequently.[2-11] In one of the more complete studies Jonas[9] recorded 5 typical cases, in all of which the myocardium and other tissues were involved by a granulomatous process characterized by tubercle-like foci with giant cells, necrosis, and mononuclear cell infiltration. In Magner's[10] report, too, giant cells were prominent among the mononuclear cells and proliferating fibroblasts in the myocardium. Saphir,[11] in his excellent review of the entire subject of myocarditis, made a necessary distinction between myocarditis with granulomatous lesions and myocarditis with a diffuse inflammatory reaction (also referred to as Fiedler's myocarditis and diffuse interstitial myocarditis). In some of the granulomatous cases to which he referred, giant cells were found; in others they were absent.

It is the purpose of this paper to present data on 2 patients with giant cell myocarditis who were necropsied at the Board of Health Laboratory, Gorgas Hospital, Ancon, Panama Canal Zone.

Case 1

A 35-year-old male mestizo suddenly became dizzy while at work, toppled over unconscious, and a few minutes later was pronounced dead. His previous medical history was entirely unknown. He was well developed and well nourished.

A complete study, including examinations of the brain and histologic sections of all organs, was done. Significant abnormalities were limited to the heart, aorta, and mediastinal lymph nodes.

Heart

Gross Examination. The heart weighed 235 gm. There was no excess pericardial fluid. The epicardium was smooth and shiny and contained little fat. The myocardium had an extraordinary appearance. Within the left ventricular wall and within the interventricular septum there

* Received for publication, June 14, 1952.

† Senior Pathologist, Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone. Now Assistant Professor of Clinical Medicine (Tropical Medicine), Cornell University Medical College.

‡ Senior Resident in Medicine, Gorgas Hospital, Ancon, Canal Zone. Now Director of Medical Research, United Fruit Company Hospital, La Lima, Honduras.

were numerous areas in which the normal red and brown parenchyma was replaced by firm, almost hard, gray tissue which was gritty when cut. These areas, interpreted as zones of fibrosis, were most prominent just beneath the epicardium on the anterior portion of the left ventricular wall. Several such patches measured approximately 1.0 by 1.0 by 0.5 cm.; others were as large as 3.0 cm. in their greatest dimension. The fibrosis was also extensive in the interventricular septum and in the left ventricular wall close to the base of the heart. No areas of softening suggestive of recent infarct were seen. No mural thrombi were encountered. The endocardium was smooth and glistening and no verrucae were present. The valve leaflets were delicate and appeared to be competent. The free edges of the aortic cusps were not thickened or rolled. The ostia of the coronary arteries were wide and patent. Both the right and left coronary arteries were moderately arteriosclerotic. The circumflex branch of the left coronary artery was small and the lumen was narrowed. The proximal first centimeter of the anterior descending branch showed moderate atherosclerosis with 50 per cent of the lumen occluded; the lumen of the remainder of this vessel was completely occluded by sclerosis and fibrosis. No recent thrombus was seen. The following were the valvular circumferences: aortic, 5.5 cm.; mitral, 10.0 cm.; tricuspid, 11.5 cm.; pulmonary, 7.0 cm. The right ventricular wall measured 0.4 cm. in thickness; the left ventricular wall measured 1.0 to 1.8 cm.; and the interventricular septum averaged 1.5 cm.

Microscopic Examination. Within the myocardium microscopic examination revealed large, diffuse and focal areas in which the muscle fibers had been completely replaced by fibrous tissue, some of which, in turn, had undergone hyaline degeneration. This fibrous tissue was extensively infiltrated with small round cells and with giant cells scattered in an apparently indiscriminate fashion (Fig. 1). The giant cells exhibited marked variation in size, shape, and appearance (Fig. 2). Many were small with relatively little eosinophilic cytoplasm and a few nuclei. Others were somewhat larger and resembled the Langhans giant cell. Still others were huge, with abundant eosinophilic cytoplasm and as many as one hundred nuclei. A few intracytoplasmic inclusions and asters were found in these giant cells but no cytoplasmic striations were demonstrated (Fig. 3). No areas of caseation necrosis were present. The large areas of fibrosis were found in sections from all portions of the heart, but were most numerous in the left ventricular wall and the interventricular septum. They were confined largely to the myocardium but in some zones had extended to the endocardium and epicardium. Sections stained with a modified Ziehl-Neelsen tech-

nic did not reveal acid-fast bacilli on prolonged search. Sections stained by a modified Levaditi technic were negative for spirochetes (controls were positive). No parasites were found. No Aschoff nodules were seen. The endocardium for the most part was thin, and showed no leukocytic infiltration. No verrucae were present. Lesions of the valves were not demonstrated.

Several sections of the two branches of the left coronary artery were essentially similar. The usual markings between adventitia and the muscle layers of the vessels were absent, and the adventitia and media were both replaced by fibrous tissue of the non-elastic variety which had undergone extensive hyalinization. The lumina of the vessels were markedly narrowed by atherosclerosis and by fibrous thickening of the wall. True proliferative endarteritis was not seen. In the connective tissue surrounding the adventitia there were several giant cells. In the media and adventitia were numerous pigment-filled macrophages. A few smaller vessels adjoining the larger ones were completely thrombosed, and fibrosis and hyalinization had occurred subsequently. Recent thrombi were not recognized. In one section taken from the smaller anterior descending branch of the left coronary artery the lumen had been converted into a narrow slit; the wall was composed of a thick mass of hyalinized connective tissue. A section of the pericardium revealed a normal lining composed of flattened mesothelial cells. In the underlying connective tissue there were found a few collections of small round cells and several giant cells of the type already described. These giant cells were distributed in an indiscriminate fashion, and bore no relation to tubercles.

Aorta

Gross Examination. Slight atherosclerosis, most prominent in the abdominal portion, was present in the aorta grossly. No longitudinal wrinkling of the ascending portion was noted. Within the adventitia of the ascending and upper thoracic portions of the aorta and in peritracheal and peri-esophageal connective tissue were a score or two of tiny, firm white nodules averaging 0.1 to 0.2 cm. in diameter. No similar nodules were found in any other organs.

Microscopic Examination. The peculiar white nodules noted grossly along the adventitia of the aorta and higher in the peritracheal region consisted of nests of compact connective tissue in which were scattered large giant cells similar to those described in the heart. Within these nests of connective tissue and around the giant cells were several diffuse and focal collections of small round cells. No areas of caseation necrosis and no gummas were seen.

Lymph Nodes

Within the parenchyma of the peritracheal and peribronchial lymph nodes were large areas of caseation necrosis, most of which were surrounded by fibrous tissue. An occasional giant cell of the Langhans type was noted in the outskirts of the zones of necrosis. These giant cells were neither numerous nor prominent. Much of the intervening parenchyma had undergone fibrosis. Anthracosis was moderate. No acid-fast bacilli were found in sections stained by a modified Ziehl-Neelsen technic.

Anatomical Diagnoses

Chronic granulomatous giant cell myocarditis, extensive; coronary arteriosclerosis with occlusion of anterior descending branch of left coronary artery; giant cell granulomas, small, of adventitia of aorta and peritracheal and peri-esophageal fascia; caseation necrosis of peritracheal and peribronchial lymph nodes; general arteriosclerosis, moderate; subarachnoid hemorrhages, small, diffuse, terminal; hemorrhages of brain, small, terminal; hydronephrosis, left kidney; focal fibrosis of kidney, probably due to nephrosclerosis; varicosities, thrombosis, phlebitis, and edema of left leg; hemorrhages into peri-adrenal fat, terminal; hyperpigmentation and scars of left leg; scar of penis, old, healed.

Cause of Death. Chronic granulomatous myocarditis, giant cell type.

*Case 2**

A 57-year-old white female died suddenly. Information received from Panama Hospital, Panama City, indicated the cause of death to be "heart block, sino-auricular standstill, Adams-Stokes syndrome." The Adams-Stokes syndrome had first manifested itself 1 month previously. Other than this, her previous medical history was unknown. Electrocardiogram was not available.

A complete necropsy, including examination of brain and histologic sections of all organs, was done.

Heart

Gross Examination. There was no excess pericardial fluid upon gross examination. The heart weighed 340 gm.; the enlargement was predominantly left ventricular. The epicardium contained a normal amount of fat. In the interventricular septum, visible both from the left ventricle and the right ventricle since it involved the entire thickness of the septum, was a firm, yellowish gray scar measuring 5.0 by 3.0 cm. This area extended upward to involve the unprotected space as a yellow fibrosed plaque which reached the base of the aortic valve.

* Necropsy performed by Dr. Wray J. Tomlinson.

In the right ventricle the lesion started just below and external to the pulmonary valve, extending under the posterior cusp of the tricuspid valve. The chambers of the heart were of average size. The valve leaflets were competent; some showed moderate sclerosis but no ulcerations or vegetations were present. The circumferences of the valves were: tricuspid, 12.0 cm.; pulmonary, 8.0 cm.; mitral, 10.5 cm.; and aortic, 8.0 cm. The left ventricular wall measured 1.7 cm. in thickness, the interventricular septum 1.2 cm., and the right ventricular wall 0.5 cm.

The coronary vessels were tortuous and palpable as sclerotic cords over the surface of the heart. Dissection revealed a complete old occlusion of the circumflex branch of the left coronary artery 5.0 cm. from its origin. There was considerable myocardial scarring in this area. The other coronary vessels showed moderate arteriosclerosis but no other complete occlusions were seen. The sino-auricular node could not be demonstrated grossly; there was no notable scarring in its usual anatomical site.

The aorta showed moderate atherosclerosis but otherwise was not remarkable. The pulmonary artery was normal.

Microscopic Examination. Microscopically, the myocardial fibers of the interventricular septum were replaced by granulomatous tissue in which were scattered monocytes, lymphocytes, epithelioid cells, and giant cells. The cells were often arranged in tubercle formation but no areas of caseation necrosis were found. Some calcific deposits were present. Although the process was most widespread in the interventricular septum, similar patches were seen in other portions of the myocardium, especially in the left ventricular wall (Fig. 4). The myocardial fibers adjacent to such involved areas showed moderate lipochrome deposition at the poles of the nuclei; some fibers were fragmented. The giant cells were of the foreign type; some, however, resembled Langhans cells. Sections stained by several methods were negative for tubercle bacilli, spirochetes, other bacteria, and parasites.

The coronary arteries showed moderate arteriosclerosis. The walls of the smaller arteries were infiltrated with plasma cells, mononuclear cells, and eosinophils. In the peri-adventitial tissues were collections of epithelioid cells and giant cells. The impression was gathered that the patches of fibrosis followed to some extent the distribution of the small coronary arteries.

Anatomical Diagnoses

Chronic granulomatous myocarditis, giant cell type; fibrosis of bundle of His; early bronchopneumonia, bilateral; generalized arteriosclerosis; congenital polycystic kidneys; arteriolar nephrosclerosis;

chronic passive congestion of liver and spleen; congenital cysts of liver; adrenal adenoma.

Cause of Death. Chronic granulomatous myocarditis, giant cell type.

Discussion

Sudden death was a feature of these 2 cases; in this respect they resemble the previously reported instances of giant cell myocarditis, in all of which there has been either rapidly progressive myocardial failure or sudden death. The predominant involvement of the left ventricular wall and of the interventricular septum was noted also in some of the previous reports, although extensive involvement of the myocardium of both auricles and ventricles apparently occurs almost as commonly.[9]

A discussion of this unusual type of myocarditis resolves itself around a consideration of possible etiologic factors. Tuberculosis is perhaps the first to suggest itself, and cannot be definitely excluded. Sarcoid is certainly a possibility.[12] Syphilis is less probable. (Serologic reactions for this disease were negative on post-mortem blood specimens of both patients. Spirochetes could not be found. The aorta was uninvolved in both cases.) Parasitic involvement by *Schizotrypanum cruzi* (Chagas' disease) was excluded.

Other diseases which might be considered but which do not conform to the pathologic picture described include giant cell sarcoma of the myocardium, rheumatic fever, periarteritis nodosa, lupus erythematosus, blastomycosis (Baker and Brian[13]), and tularemia (Lillie[14]).

In view of the distribution of the lesions and the involvement of the arteries there is a possibility that the process represents a peculiar reaction to coronary arterial disease.

Summary

In two instances of sudden death the histologic features of giant cell myocarditis were found. There was no caseous necrosis. Appropriate examinations for the demonstration of infective causal agents gave negative results.

The suggestion is made (on rather inadequate evidence) that giant cell myocarditis may represent a peculiar reaction to coronary arterial disease.

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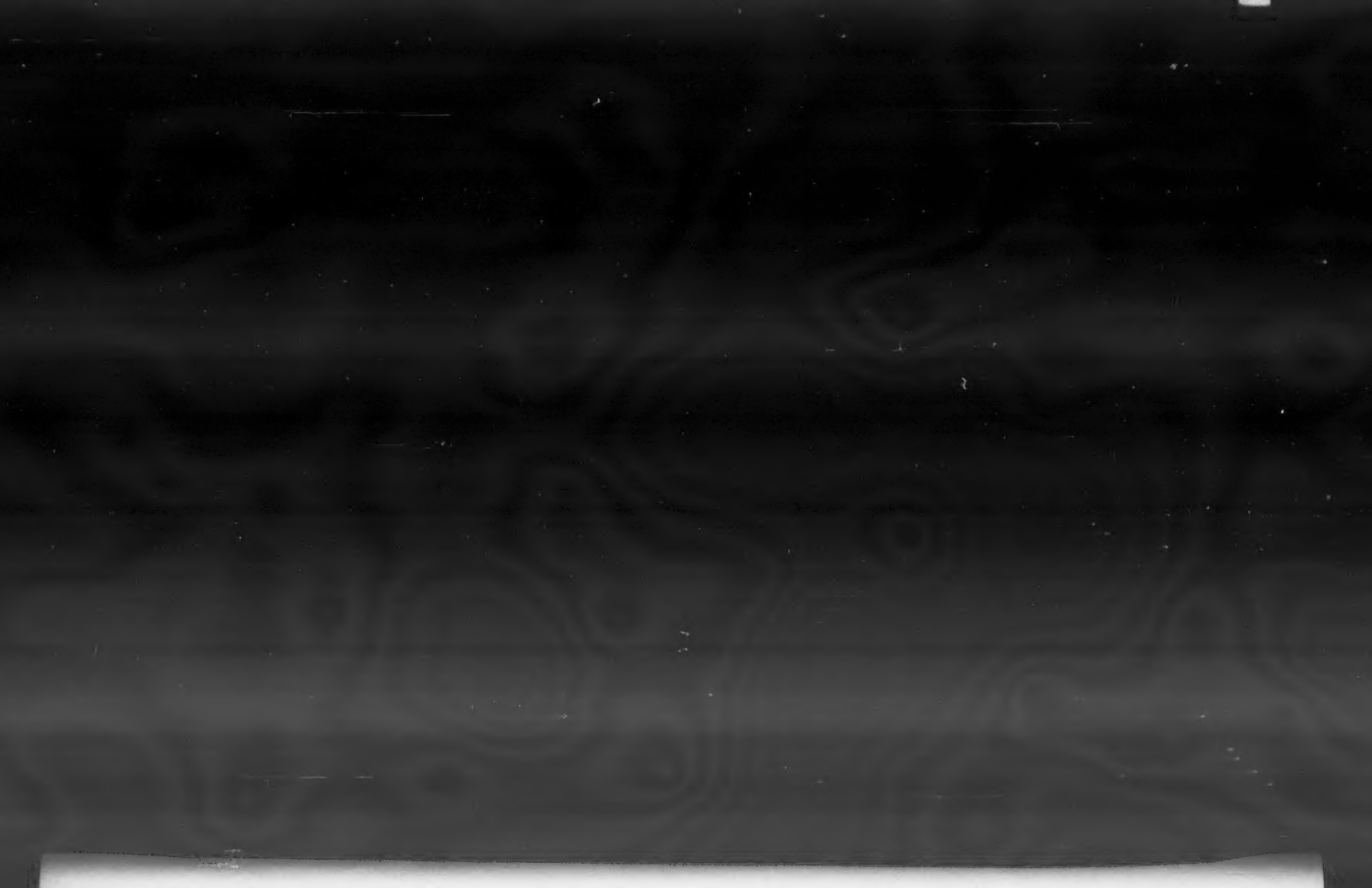
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 172

FIG. 1. Case 1. Section of interventricular septum showing fibrosis, infiltration of lymphocytes, and many giant cells. × 60.

FIG. 2. Case 1. Section of left ventricular wall showing types of giant cells. × 60.



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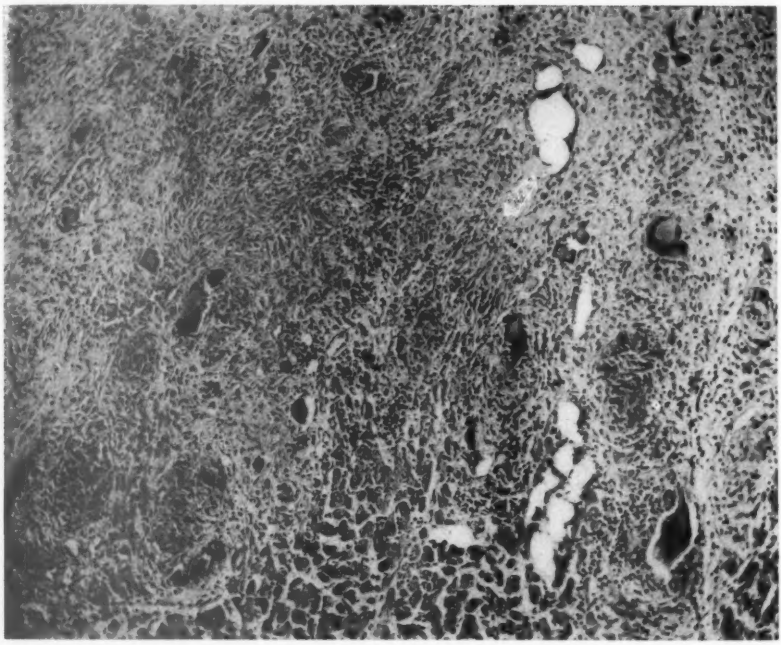

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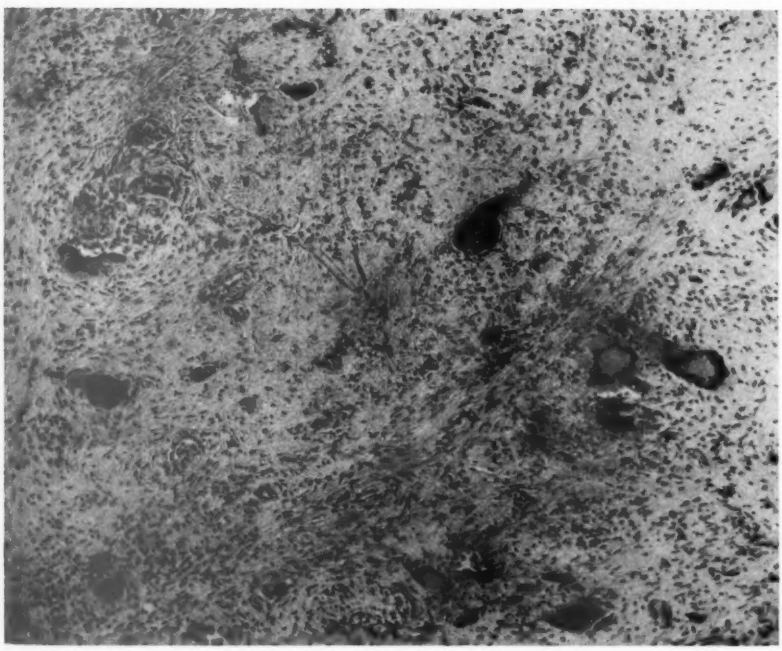


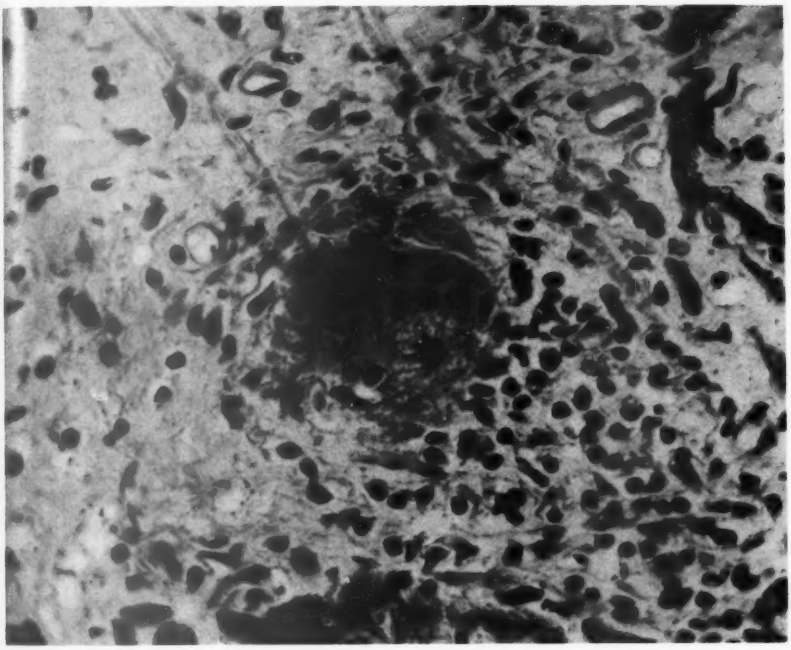
Kean and Hoekenga Giant Cell Myocarditis

PLATE 173

FIG. 3. Case 1. Aster, star-shaped inclusion body in this giant cell. × 450.

FIG. 4. Case 2. Patches of giant cell myocarditis in left ventricular wall. × 60.

3



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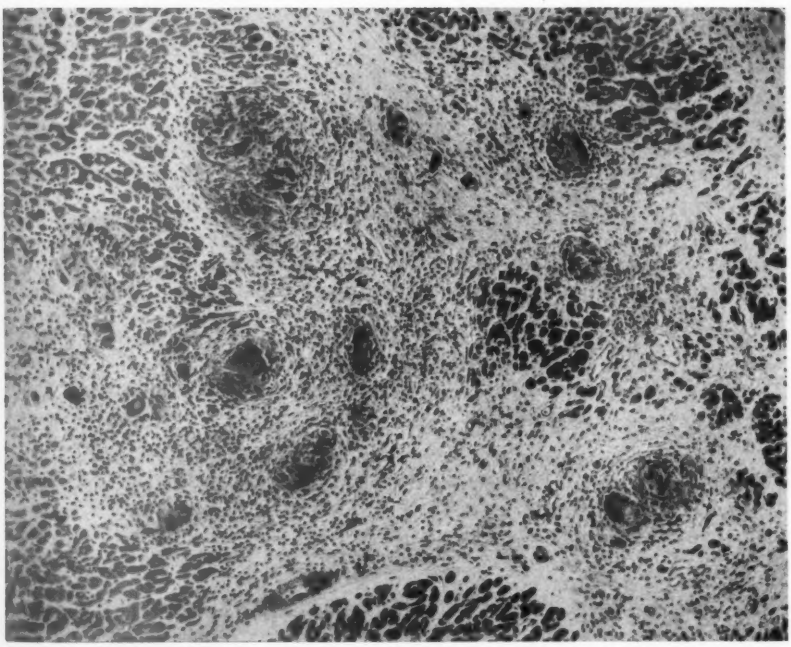

Kean and Hoekenga Giant Cell Myocarditis

CALCIFICATION IN INTRACRANIAL NEOPLASMS *

FRANKLIN MARTIN, JR., M.D., and LLOYD J. LEMMEN, M.D.

(From the Laboratory of Neuropathology of the Neuropsychiatric Institute, the Department of Pathology, and the Section of Neurological Surgery of the Department of Surgery, University of Michigan, Ann Arbor, Mich.)

This study of the histopathologic patterns of calcification was undertaken to provide a basis for the interpretation of the roentgenographic appearance of the shadows cast by these deposits, since the presence of calcium salts in intracranial neoplasms affords an accurate localization of such lesions in routine roentgenograms.

MATERIAL AND METHODS

The histologically verified intracranial tumors of all types treated in the Department of Surgery during the period 1930–1950 were reviewed. The series consisted of 1577 neoplasms.

Our classification of the material generally follows that of Bailey and Cushing,[1] except that spongioblastoma polare has been grouped with the astroblastomas. For purposes of comparison with the series of other authors an attempt was made also to regroup the tumors within the concept of gliomas introduced by Kernohan.[2,3]

In cases difficult to classify, additional sections and special stains were used. The stains were hematoxylin and eosin, Mallory's phosphotungstic acid hematoxylin, Heidenhain's azan, Nissl's azure eosin, Perdrau's, and in the more recent material, Hortega's silver carbonate methods.

Gross morphology is not reviewed in this report as it has been considered by others.[4-7]

HISTOLOGIC PATTERNS OF CALCIFICATION

For the sake of classification, calcium deposits were grouped according to the occurrence of the dominant histologic pattern, and were divided into four types.

Pattern I. The type of calcification most frequently encountered occurred within blood vessel walls. This form, designated as pattern I, shows small globules or calcospherites within the adventitia or media of the smaller vessels and the endothelium of capillaries. In early stages these calcospherites appear as spherical or coalesced globules marking the course of the involved vessel. In more advanced stages the vessels are entirely calcified, have no lumina, and appear as solid cylinders (Figs. 1 to 4). In larger vessels, the calcium deposits appear

* Received for publication, February 26, 1952.

as small, spherical globules within the media. Coalescence of these globules in later stages converts the vessel into a rigid tube which appears in cross section as a ring of calcium.[8]

Pattern II. When the changes described as pattern I involve adjacent blood vessels whose zones of necrosis overlap (Fig. 13), concentric layers of calcium marking the former site of each vessel result in a conglomerate mass of calcium. This is designated as pattern II. It superficially resembles pattern III.

Pattern III. In pattern III calcium salts are deposited in an area of old hemorrhage or necrosis. A solid layer of calcium having irregular borders and no discernible vascular pattern is seen (Figs. 7 and 8).

Pattern IV. Pattern IV consists of several types: (a) globules of calcium as seen within tumor cells (Figs. 15 and 16), (b) calcific changes within the stroma of a tumor or its capsule (Figs. 9 to 11), (c) calcific changes at the periphery of a tumor, but not related to types (a) or (b). Under certain conditions, as, for instance, the presence of psammoma bodies, it was not always possible to determine whether the calcification occurred within blood vessel walls or within tumor elements.

Pathogenesis of Calcification

Chemical studies were not undertaken. Fully developed calcification gives no indication as to its origin, but many stages of the development of calcified areas were observed. In patterns I and II, uninvolved vessels in the vicinity of those calcified showed swelling and hyalinization of their walls. By the time calcium droplets could be seen, nuclear structure was no longer observed. It thus appeared that necrosis was associated with the deposition of calcium, at least in some of the vessels.

The antecedent necrosis in pattern III was often evident even after calcification was advanced. Ischemic or hemorrhagic infarction appeared to be the basis of the necrosis. In pattern IV, psammoma bodies were frequently associated with hyalinization and calcified cells were obviously non-viable. The earlier stages of these types were not observed. An explanation for the interstitial calcium deposits was not apparent.

Incidence of Calcification

On histologic examination, calcification was found in 207 or 13.1 per cent of 1577 intracranial tumors. Pattern I was most common and occurred 93 times with the highest incidence in the oligodendrogliomas (15 of 34 cases). Pattern IV was seen in 85 tumors, chiefly craniopharyngiomas (39 of 58 cases). Pattern III was present in 20

tumors of which 5 were ependymomas (5 of 55 ependymomas). Pattern II was least often represented, occurring only 9 times in the series, with a tendency to be associated with glioblastoma multiforme. These figures, however, are not statistically significant (Tables I and II).

Location of Calcium Deposits

Tumors of the Cerebral Hemispheres

Supratentorial gliomas of the cerebral hemispheres showed calcium deposits in 12.5 per cent, the incidence varying with the tumor type, being highest in the oligodendrogliomas (53 per cent) and high, also, in the ependymomas (32.2 per cent). The most common type of calcification was pattern I, which occurred most frequently in oligodendrogliomas (Fig. 2 and Table I). In the category of neuroblastic tumors, which include the neurocytomas and neuroblastomas, calcification was seen in 3 of 7 cases, one case showing calcification of blastomatous neurons (Figs. 15 and 16). Among the gliomas limited primarily to one lobe (determined by necropsy and operative findings), calcification was found more commonly in the frontal region, usually within oligodendrogliomas or astrocytomas (Table III).

The largest number of calcified gliomas (reclassified according to Kernohan[2]) occurred in astrocytomas, grades 2 and 3 (25 of 284 tumors) (Tables V and VI).

The ependymomas showed calcification in 10 cases of which 8 were classified histologically[9] as the cellular type and 2 as the myxopapillary type. One of the two papillomas of the choroid plexus of a lateral ventricle was found to have calcium deposits.

Calcium deposits were present in 40, or 18.6 per cent, of 215 meningiomas; 34 calcified tumors belonged to the supratentorial group. Among these cases there were 28 of meningotheliomatous, 4 of psammomatous, and 2 of lipomatous type.[6,10] Calcification in meningiomas occurred typically in the hyalinized vessels of the whorl formations (Fig. 14). In this group the calcified tumors were located more frequently in the frontal and parasagittal regions (Tables III and IV).

Tumors in the Region of the Third Ventricle

Among the tumors of the region of the third ventricle occur the gliomas arising primarily in the basal ganglia and thalamus. Although glioblastoma multiforme occurred most frequently in this region, calcification in these structures was limited to astrocytomas, oligodendrogliomas, and ependymomas. Calcification was observed in one tumor of the optic nerve (spongioblastoma polare). None of the colloid cysts

TABLE I

Microscopic Calcification in Supratentorial Tumors (1930–1950)

		Sex		Age (years)					Average age (years)	Average duration of preoperative symptoms (months)	No. operated upon	No. necropsied	No. receiving x-ray therapy	5-yr. survival	No. tumors of various patterns of calcification				No. with calcification visible roentgenologically
		M	F	0–15	16–30	31–40	41–50	Over 50							I	II	III	IV	
Astrocytoma	Total.....165	109	56	14	45	51	37	18	34	20	163	37	7	14					
	No. calc... 24	15	9	1	7	8	6	2	35	21	24	3	2	5	16	0	3	5	14
Astroblastoma	Total..... 31	22	9	7	4	4	7	9	37	12	29	12	3	6					
	No. calc... 3	3	0	0	0	1	1	1	51	5	2	1	1	1	3	0	0	0	0
Glioblastoma multiforme	Total.....398	263	135	14	39	66	138	141	45	7	388	119	21	4					
	No. calc... 25	16	9	1	8	2	6	8	40	13	23	9	3	0	12	4	3	6	4
Oligodendroglioma	Total..... 34	19	15	3	7	5	7	12	39	20	34	10	0	8					
	No. calc... 18	9	9	2	4	2	3	7	35	10	18	7	0	7	15	0	1	2	14
Ependymoma	Total..... 31	15	16	13	8	5	3	2	22	19	31	6	6	11					
	No. calc... 10	5	5	5	3	2	0	0	16	46	10	2	2	6	3	2	4	1	6
Neuroblastic tumors	Total..... 7	2	5	1	3	1	2	0	31	41	7	0	0	0					
	No. calc... 3	0	3	1	2	0	0	0	20	25	2	0	0	0	1	1	0	1	2
Papilloma of choroid plexus	Total..... 3	1	2	1	2	0	0	0	16	36	3	1	1	1					
	No. calc... 1	0	1	0	1	0	0	0	20	1	1	0	0	1	1	0	0	0	0
Colloid cyst	Total..... 8	4	4	1	5	1	1	0	25	16	7	3	0	2					
	No. calc... 0																		
Tumors of pineal region	Total..... 19	13	6	9	6	2	0	2	23	11	18	15	4	2					
	No. calc... 8	5	3	4	2	0	0	2	29	15	8	7	1	1	6	0	1	1	7
Teratoma	No. calc... 4	4	0	4	0	0	0	0	7	1	3	3	1	1	0	0	0	4	0
Pituitary tumors	Total..... 97	50	47	3	19	24	33	18	41	28	96	20	27	33					
	No. calc... 5	2	3	0	2	2	0	1	36	9	4	3	2	1	1	0	2	2	1
Craniopharyngioma	Total..... 58	33	25	28	9	9	8	4	22	19	58	14	0	13					
	No. calc... 39	23	16	22	6	3	5	3	10	17	39	12	0	7	0	0	0	39	30
Optic nerve tumors	Total..... 16	8	8	14	0	0	0	2	15	21	16	5	2	7					
	No. calc... 1	1	0	1	0	0	0	0	15	72	1	1	0	0	0	0	0	1	0
Meningioma	Total.....193	78	115	3	20	33	73	64	45	27	186	32	2	67					
	No. calc... 34	9	25	1	1	4	14	14	50	32	31	6	0	14	16	1	1	16	19
Sarcoma	Total..... 23	12	11	4	3	4	9	3	37	8	23	4	5	2					
	No. calc... 2	2	0	0	0	0	2	0	46	42	2	0	1	1	0	0	1	1	2
Metastatic tumors	Total..... 72	48	24	0	4	11	21	36	49	6	72	9	1	0					
	No. calc... 1	1	0	0	0	0	1	0	45	9	1	0	0	0	1	0	0	0	0

Metastatic tumors	Total..... 72	48	24	0	4	11	21	36	49	6	72	9	1	0							
	No. calc... 1	1	0	0	0	0	1	0	45	9	1	0	0	0	1	0	0	0	0		

TABLE II

Microscopic Calcification in Posterior Fossa Tumors (1930–1950)

		Sex		Age (years)					Average age (years)	Average duration of preoperative symptoms (months)	No. operated upon	No. necropsied	No. receiving x-ray therapy	5-yr. survival	No. tumors of various patterns of calcification				No. with calcification visible roentgenologically
		M	F	0–15	16–30	31–40	41–50	Over 50							I	II	III	IV	
Cerebellar glioma	Total..... 101	44	57	63	25	6	0	7	14	10	98	12	6	44					
	No. calc... 12	7	5	6	5	1	0	0	17	17	12	3	0	6	9	0	2	1	4
Brain stem glioma	Total..... 48	26	22	24	12	5	3	4	21	10	29	36	2	1					
	No. calc... 2	2	0	2	0	0	0	0	13	1	2	1	0	0	2	0	0	0	0
Ependymoma	Total..... 24	13	11	17	1	2	3	1	15	5	22	7	8	2					
	No. calc... 3	2	1	3	0	0	0	0	4	3	3	3	1	0	1	1	1	0	1
Medulloblastoma	Total..... 72	41	31	58	8	6	0	0	11	6	69	19	46	6					
		32	26	58					7	4	55	16	34	4					
	No. calc... 4	4	0	4	0	0	0	0	9	4	4	0	3	0	2	0	1	1	0
Papilloma of choroid plexus	Total..... 6	6	0	0	0	2	4	0	47	9	6	0	1	0					
	No. calc... 2	2	0	0	0	0	2	0	47	13	2	0	0	0	1	0	0	1	0
Acoustic tumors	Total..... 93	38	55	0	18	17	32	26	42	33	91	29	0	22					
	No. calc... 0																		
Hemangioblastoma	Total..... 22	17	5	3	4	5	8	2	36	11	22	7	1	3					
	No. calc... 0																		
Meningioma	Total..... 22	13	9	0	0	4	9	9	49	23	21	4	0	3					
	No. calc... 6	4	2	0	0	1	1	4	55	28	5	1	0	0	3	0	0	3	0
Sarcoma	Total..... 11	9	2	4	7	0	0	0	16	11	11	1	7	0					
	No. calc... 0																		
Metastatic tumors	Total..... 23	15	8	0	1	6	9	7	48	7	23	6	0	0					
	No. calc... 0																		

Total, 422; number with calcification, 29.

of the third ventricle, papillomas of the choroid plexus of the third ventricle, or meningiomas of the tuberculum sellae were calcified (Table III).

Of 58 "craniopharyngiomas," 39 or 67.2 per cent were calcified. Of these 28 were designated as adamantinoma, 22, or 78.6 per cent, of which contained calcium characteristically adjacent to the epithelial

TABLE III

The Occurrence of Calcification as to Location (Operative or Necropsy Findings)

		Involving primarily one cerebral lobe				Involving more than one cerebral lobe
		Frontal	Parietal	Temporal	Occipital	
Astrocytoma	Total	51	20	19	0	60
	No. calc.	8	5	2	0	9
Astroblastoma	Total	8	2	8	1	8
	No. calc.	2	0	0	0	1
Glioblastoma multiforme	Total	72	55	76	6	167
	No. calc.	4	3	7	0	10
Oligodendroglioma	Total	10	4	0	1	15
	No. calc.	6	2	0	1	8
Ependymoma	Total	4	8	8	1	7
	No. calc.	2	1	2	1	3
Meningioma	Total	87	32	40	2	16
	No. calc.	15	7	5	1	5

Tumors of third ventricle and adjacent structures

	Total	No. calc.
Craniopharyngioma	58	39
Pituitary tumors	97	5
Meningioma, tuberculum sellae	4	0
Optic nerve tumors	16	1
Colloid cyst	8	0
Papilloma of choroid plexus	1	0
Tumors of pineal region	19	12
Basal ganglia and thalamus		
Glioblastoma multiforme	26	0
Astrocytoma	21	2
Oligodendroglioma	6	1
Ependymoma	5	2

cells (Fig. 9). In tumors of this group classified as "epithelial" 16 of 28 cases, or 57.1 per cent, were calcified, the calcium being in either the stroma of the tumor (Fig. 11) or in the cystic spaces (Fig. 10). In this group were one teratoid tumor which showed no calcium deposits and one suprasellar teratoma which contained cartilage and bone.

Of 19 tumors of the pineal region 12, or 63.2 per cent, were calcified.

Calcium deposits were found in 7 pinealomas and in one pinealoblastoma. In 4 teratomas of the pineal region the calcium was deposited in cartilage and bone. The most frequent pattern was that of type I, which was present in 6 pinealomas (Fig. 4).

Pituitary neoplasms were classified as chromophobic in 92 cases and eosinophilic in 5. Calcium was found in 5, or 5.4 per cent, of those of chromophobe type (Fig. 12); of these 2 were of pattern III (Table I).

Tumors of the Posterior Fossa

The gliomas of the posterior fossa showed predominance of pattern I with less selectivity as to tumor type. In 94 astrocytomas of the cerebellum, calcification occurred 12 times, or in 12.8 per cent; and in 72 medulloblastomas, 4 times, or 5.6 per cent (Figs. 5 and 6). In

TABLE IV
Locations of Supratentorial Meningiomas

	No. of tumors	No. calc.
Parasagittal		
Frontal	35	6
Parietal	13	4
Frontal	35	3
Parietal	19	3
Temporal	5	0
Occipital	2	1
Fronto-parietal	8	4
Temporo-parietal	4	1
Parieto-occipital	4	0
Sphenoid ridge	36	5
Olfactory groove	18	6
Tuberculum sellae	4	0
Interventricular	4	0
Gasserian ganglion	6	1
Total	193	34

48 astroblastomas (spongioblastoma polare of Bailey) which involved the brain stem, 2 were calcified and both showed pattern I. With reclassification of the cerebellar gliomas, calcification was found most frequently in astrocytoma, grade 2[3] (Table VI).

Of 24 ependymomas of the posterior fossa there were calcium deposits in 3, classified histologically as epithelial type (2 cases) and cellular type (1 case) (Figs. 7 and 8). Two examples of papilloma of the choroid plexus in the fourth ventricle had calcium deposits.

In the posterior fossa, 6, or 27.3 per cent, of the 22 meningiomas were calcified; 5 were of the meningotheliomatous and one of the

psammomatous type. In this region two major tumor groups, acoustic schwannoma (93 cases) and hemangioblastoma of the cerebellum (22 cases), were without calcification (Table II).

Metastatic Tumors

Of 95 examples of surgically treated metastatic tumors, only one, and that from bronchogenic carcinoma, was calcified (pattern I). This group was heterogeneous, with 72 tumors in the supratentorial region

TABLE V

Supratentorial Astrocytomas (Reclassified)

Grade of tumor malignancy	Number of patients	Number of cases with calcification	Average age (years)	Average preoperative duration of symptoms (months)	Average postoperative survival (months)
1	24 (32)	3	31 (33.9)	18.5 (20.8)	28 (73.6)
		3	29	18	50
2	80 (38)	12	33.6 (38.2)	18.2 (11.2)	27 (23.8)
		12	30	18.2	48.5
3	143 (37)	13	45 (40.4)	11.6 (11.8)	10.2 (11.5)
		13	43	11	22.4
4	37 (54)	3	41.7 (42.6)	5.2 (7.3)	12.3 (6.6)
		3	25	8.3	23.3
Total	284				

This material is based on cases who survived over 1 month after operation. Many of these patients are still living. Figures in parentheses are reported figures of Kernohan *et al.*[2,3]

and 23 in the posterior fossa. In the supratentorial group there were 22 tumors from bronchogenic carcinoma, 10 melanoblastomas, 5 hypernephromas, 5 from cutaneous structures, 3 from breast, 2 from oral mucosa, and one each from cervix, rectum, and stomach. One case was a chorio-epithelioma. In 21 cases the site of the primary tumor remained clinically and pathologically obscure.

Of the 23 metastatic tumors in the posterior fossa 8 were of pulmonary origin, 3 hypernephromas, 2 hemangiosarcomas (from ilium and petrous bone), one each from breast, stomach, rectum, rectosigmoid, nasopharynx, ovary, and uterus. In 3 cases the primary site was not established.

CALCIFICATION WITHIN AGE GROUPS

In the age group 0 to 15 years the incidence of calcification was highest in craniopharyngioma (22 of 28, or 78.6 per cent) (Table I). The most frequently calcified cerebral glioma was ependymoma with 5 of 13 examples, or 38.5 per cent. Eight, or 89 per cent, of 9 tumors

of the pineal region were calcified (Table I). Calcification occurred in 9.5 per cent of the astrocytomas of the cerebellum and in 6.9 per cent of medulloblastomas in the posterior fossa tumors of the same age group (Table II).

The gliomas of the adult group (older than 15 years) did not show calcification characteristic of any tumor type or age group. The supratentorial gliomas, glioblastomas, and oligodendrogliomas were more

TABLE VI

Cerebellar Astrocytomas (Reclassified)

Grade of tumor malignancy	Number of patients	Number of cases with calcification	Average age (years)	Average preoperative duration of symptoms (months)	Average postoperative survival (months)
1	32 (109)	3	12.1	9.6 (13.9)	89 (78)
		3	19	24	50.5
2	35 (14)	8	17	17.5 (11.7)	75 (74.6)
		8	18.5	19.3	62
3	9 (8)	1	24	5.5 (8.5)	30 (84)
		1	10	7.5	20
4	0 (0)				
Total	76				

This material is based on cases who survived over 1 month after operation. Many of these patients are still living. Figures in parentheses are reported figures of Kernohan *et al.*[2,3]

frequently calcified after age 50. Calcification of the meningiomas was more frequent after age 40, although this trend was not statistically significant (Table I).

Calcification in Relation to Sex

The gliomas were more common in males while meningiomas were proportionately more frequent in females. No significant difference with regard to sex was noted in the occurrence of calcification (Table I).

Preoperative Duration of Symptoms in Relation to Calcification

For glioblastoma multiforme the duration of preoperative symptoms was 13 months for calcified tumors and 7 months for the total group. In ependymoma it was 46 months for calcified tumors and 19 months for the total group. However, it was shorter in calcified oligodendrogliomas in which it was 10 months for calcified neoplasms and 20 months for the total group. For the other types of tumor there was no significant difference (Tables I and II).

Survival with Relation to Calcification

A survival period of 5 years was used as an index for prognosis. In the supratentorial group, patients with calcified ependymoma, astrocytoma, and oligodendroglioma had a significantly longer survival period than corresponding cases without calcification (Table I). In the posterior fossa no significant relationship was noted between the survival periods of patients with calcified tumors and the total group (Table II).

Roentgenographic Calcification

Calcification was demonstrated microscopically in 207, or 13.1 per cent, of the 1577 tumors. Of 192 tumors in which calcification was found microscopically, the roentgenologist reported calcification radiographically in 104 or 54.2 per cent. The supratentorial tumors having microscopic calcification had confirmatory radiologic changes in 59.6 per cent (99 of 166). Tumors of the posterior fossa showing microscopic calcium salts had this finding confirmed by x-ray in 19.2 per cent (5 of 26) (Tables I and II).

Discussion

Although no attempt was made to correlate microscopic calcification with roentgenographic shadows, a consideration of the three major histologic patterns, as demonstrated in this study, may occasionally aid in the interpretation of the roentgenogram. The first pattern gives rise to a flocculent or worm-like shadow resembling the vascular pattern as it appears on the arteriogram.[11] The granules correspond to the cross section and the parallel radiations to the longitudinal view of the calcified vessels. The second and third patterns may appear on the roentgen film either as a single large shadow or as a solid sheet of calcium. Pattern IV includes calcification of several types, and does not cast a characteristic shadow.

Since a large proportion of this study was based upon biopsy material, calcification noted on the roentgenogram[12] was not necessarily represented in microscopic sections of the tissue and, conversely, the minute amounts noted microscopically were not necessarily visible radiographically because of their size and position. It is reasonable to assume that minute calcium fragments seen microscopically in medulloblastoma would not be visible through the dense bone of the posterior fossa. No systematic study of the size, density, and distribution of calcium salts necessary to cast a shadow in roentgenograms of the skull is available from the literature.

Although radiologic diagnosis of the neoplastic type from the pattern of the calcium salts has not been attempted, this pattern, combined with pertinent clinical information, may suggest the true nature of the lesion. This information becomes useful in the interpretation of roentgenograms of patients with symptoms of space-occupying lesions when calcium deposits are visible. In children, sheets or masses of supratentorial calcium in the roentgenogram suggest the presence of an ependymoma.[13] Calcification in the suprasellar region in children is highly suggestive of craniopharyngioma (Figs. 21 and 22). From our material no definite histopathologic diagnosis could be made when calcification occurred in the pineal region of children.[14] Calcification in neoplasms visualized by x-ray in the posterior fossa of children is most suggestive of astrocytoma or ependymoma of the cerebellum (Figs. 24 and 25).

In adults, roentgenographic correlation with the histologic tumor type is more difficult. However, in the middle-aged group, calcified tumors of the frontal region are most likely to be astrocytoma or oligodendroglioma. In those patients over the age of 50, oligodendroglioma is the most probable lesion (Fig. 18). Calcified lesions of the parasellar region are more prone to be craniopharyngioma (Figs. 21 and 22) than a primary neoplasm of the pituitary gland (Fig. 23). Calcification in close association with the dura or falx may suggest the diagnosis of meningioma[13] (Figs. 19 and 20). This should not be confused with the physiologic calcification which occurs commonly at these sites. Elsewhere, calcification in the supratentorial region may be of any histopathologic type (Fig. 17).

Conclusions

Each of 1577 verified tumors of the central nervous system was studied for microscopic calcification. Calcification was found in 207 or 13.1 per cent.

Four histologic patterns of calcification were differentiated in this study and three of these occurred frequently.

When calcification is visible in the roentgenogram, a consideration of these microscopic patterns and their locations, when correlated with clinical data, may in certain instances suggest the histologic structure.

In this series an insufficient number of cases was examined following roentgen therapy to ascertain any relationship between irradiation and the occurrence of calcification in particular tumor types.

Calcification occurred in our series in every major tumor group except the schwannomas and hemangioblastomas.

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DESCRIPTION OF PLATES

PLATE 174

FIG. 1. Supratentorial glioblastoma multiforme with calcified blood vessels, pattern I. Hematoxylin and eosin stain. × 155.

FIG. 2. Oligodendroglioma with beginning confluence of calcified blood vessels, pattern I. Hematoxylin and eosin stain. × 130.

FIG. 3. Cerebral astrocytoma with calcified blood vessel wall, pattern I. Hematoxylin and eosin stain. × 155.

FIG. 4. Calcified blood vessel in pinealoma, pattern I. Hematoxylin and eosin stain. × 155.

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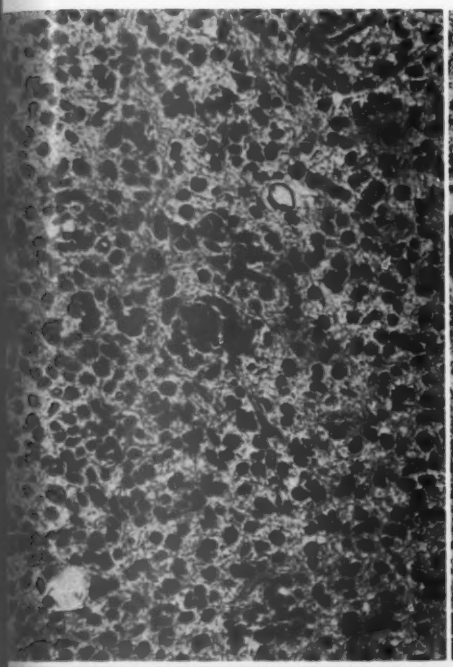

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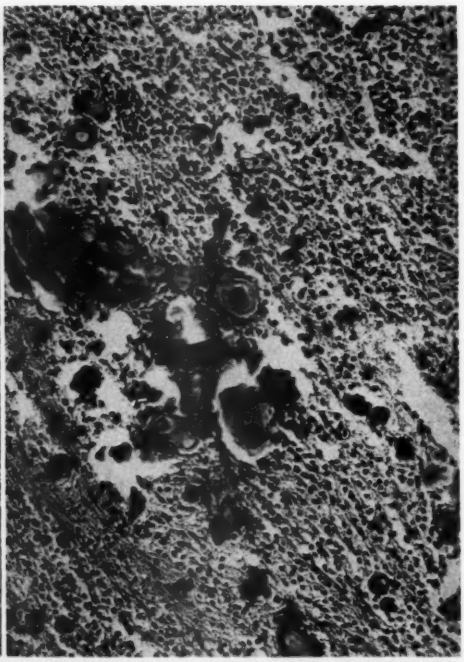

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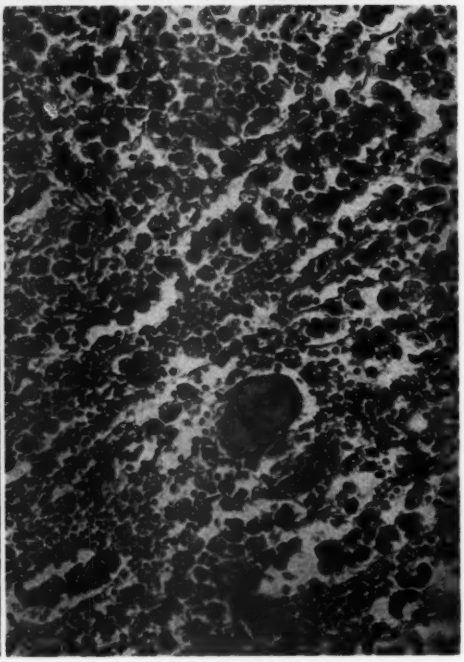

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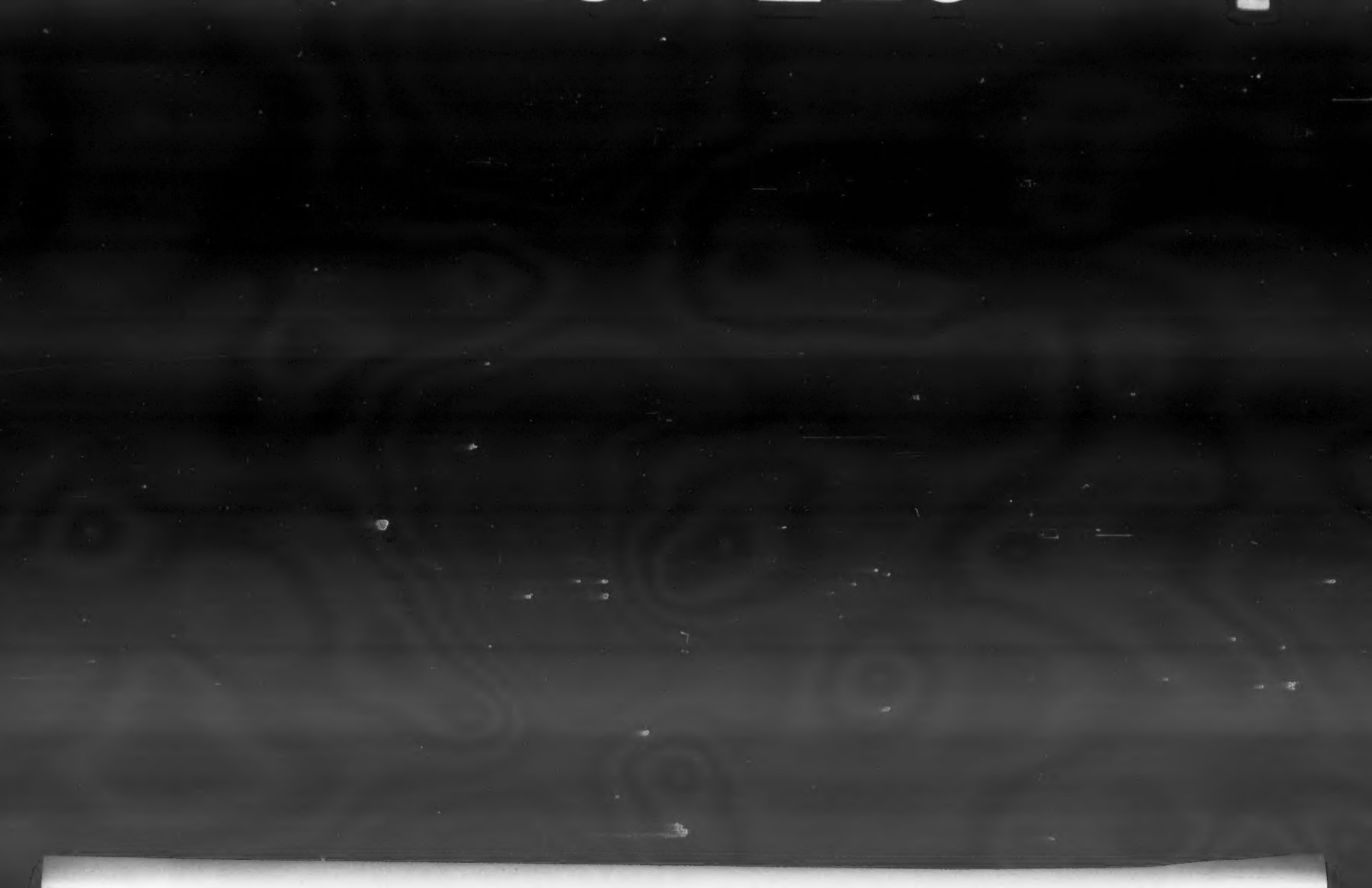
PLATE 175

FIG. 5. Medulloblastoma with calcification of pattern I. Hematoxylin and eosin stain. × 140.

FIG. 6. Cerebellar astrocytoma with calcification of pattern II. Hematoxylin and eosin stain. × 150.

FIG. 7. Epithelial ependymoma from the posterior fossa, showing calcification of pattern III in an area of necrosis. Hematoxylin and eosin stain. × 125.

FIG. 8. From the same ependymoma as Figure 7, showing another area of calcification of pattern III. Hematoxylin and eosin stain. × 125.



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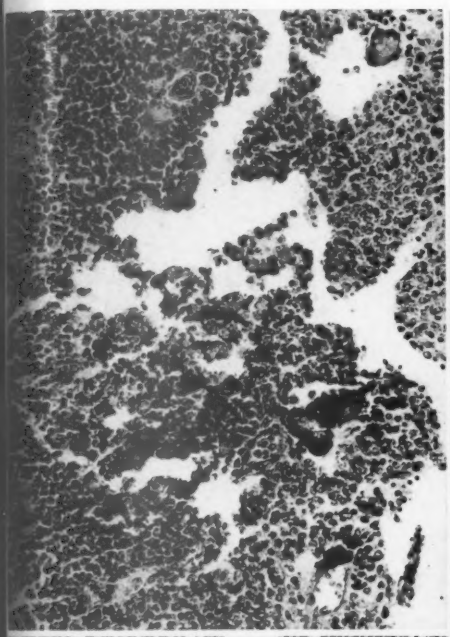

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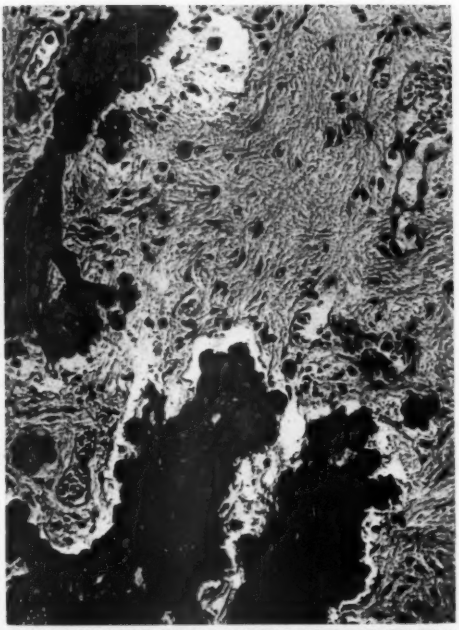

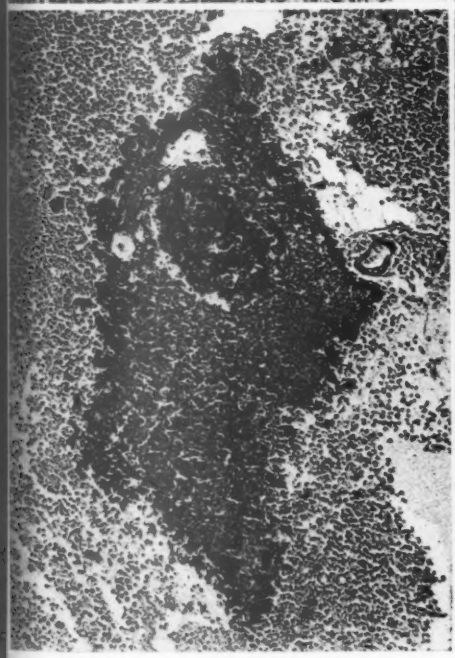

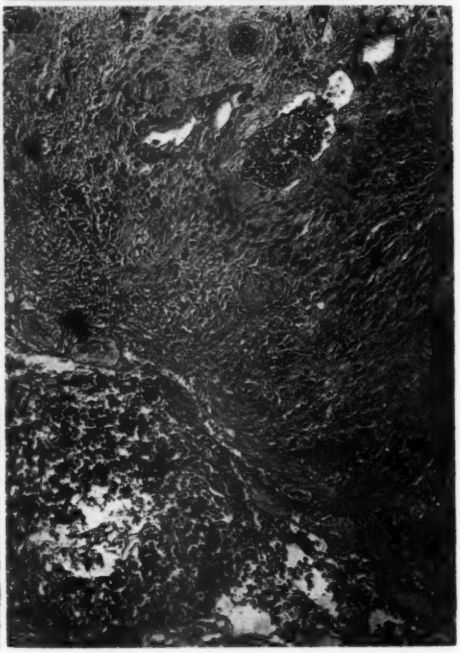

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Martin and Lemmen Calcification in Intracranial Neoplasms

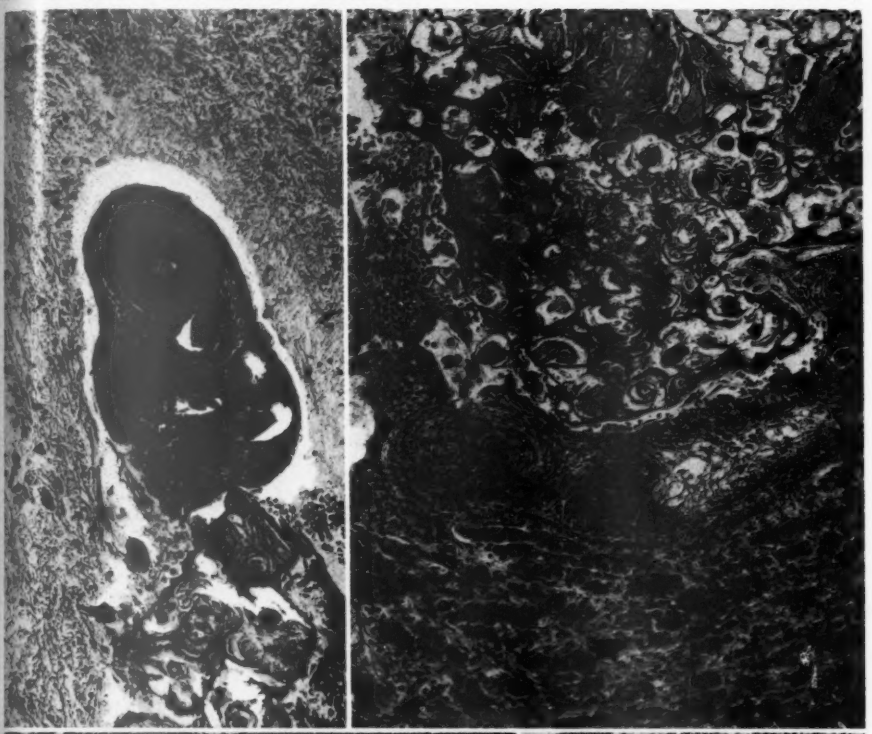
PLATE 176

FIG. 9. Craniopharyngioma with calcification surrounded by epithelial cells, pattern IV. Hematoxylin and eosin stain. × 130.

FIG. 10. Craniopharyngioma adjacent to pituitary gland with calcification of pattern IV. Hematoxylin and eosin stain. × 145.

FIG. 11. Craniopharyngioma with sheets of calcification of pattern IV. Hematoxylin and eosin stain. × 130.

FIG. 12. Chromophobe adenoma of the pituitary gland showing calcification in blood vessel, pattern I. Hematoxylin and eosin stain. × 145.



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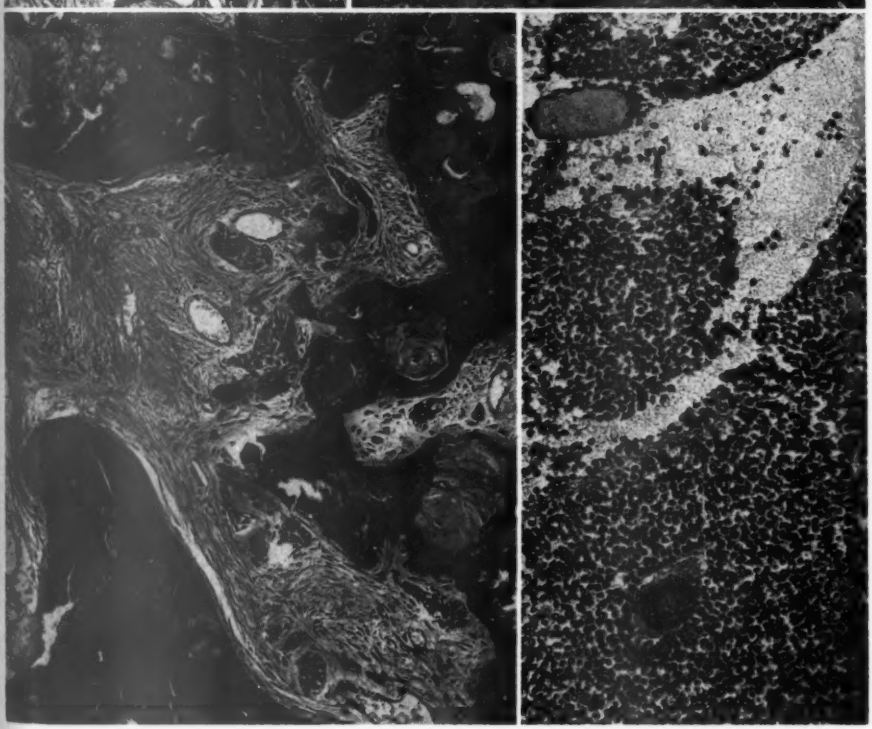

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PLATE 177

FIG. 13. Meningotheliomatous meningioma of the olfactory groove, showing confluence of calcified blood vessels of pattern II. Hematoxylin and eosin stain. × 125.

FIG. 14. Meningioma of the choroid plexus showing calcification of pattern I. Hematoxylin and eosin stain. × 132.

FIG. 15. Neurocytoma with living ganglion cells (below) and calcified ganglion cells (above), pattern IV. Hematoxylin and eosin stain. × 132.

FIG. 16. Neurocytoma with multiple calcified ganglion cells, pattern IV. Hematoxylin and eosin stain. × 118.

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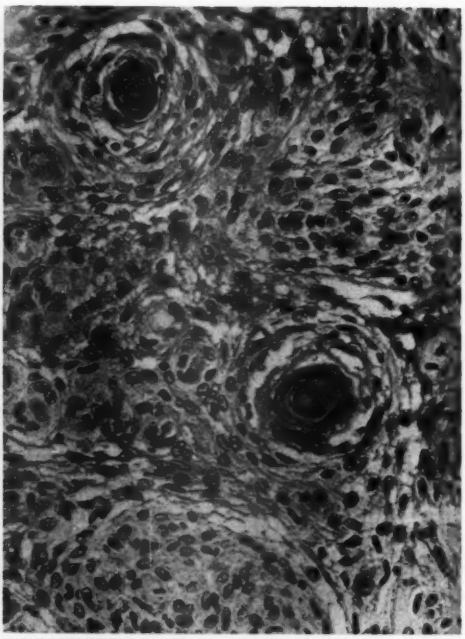

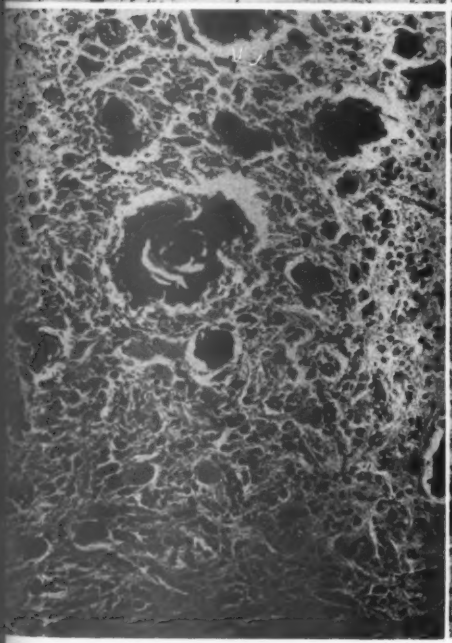

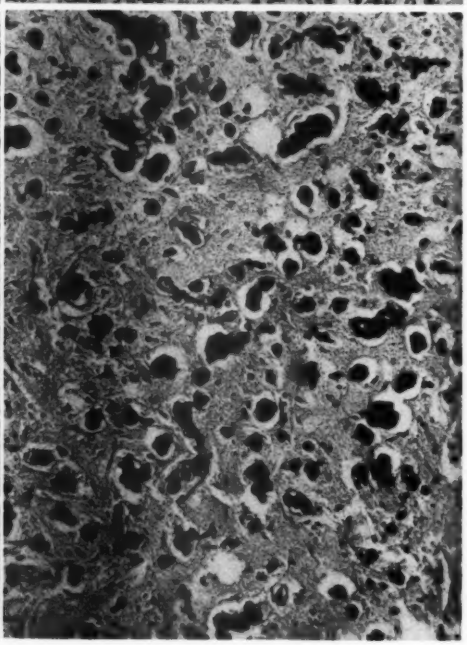

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Martin and Lemmen

Calcification in Intracranial Neoplasms

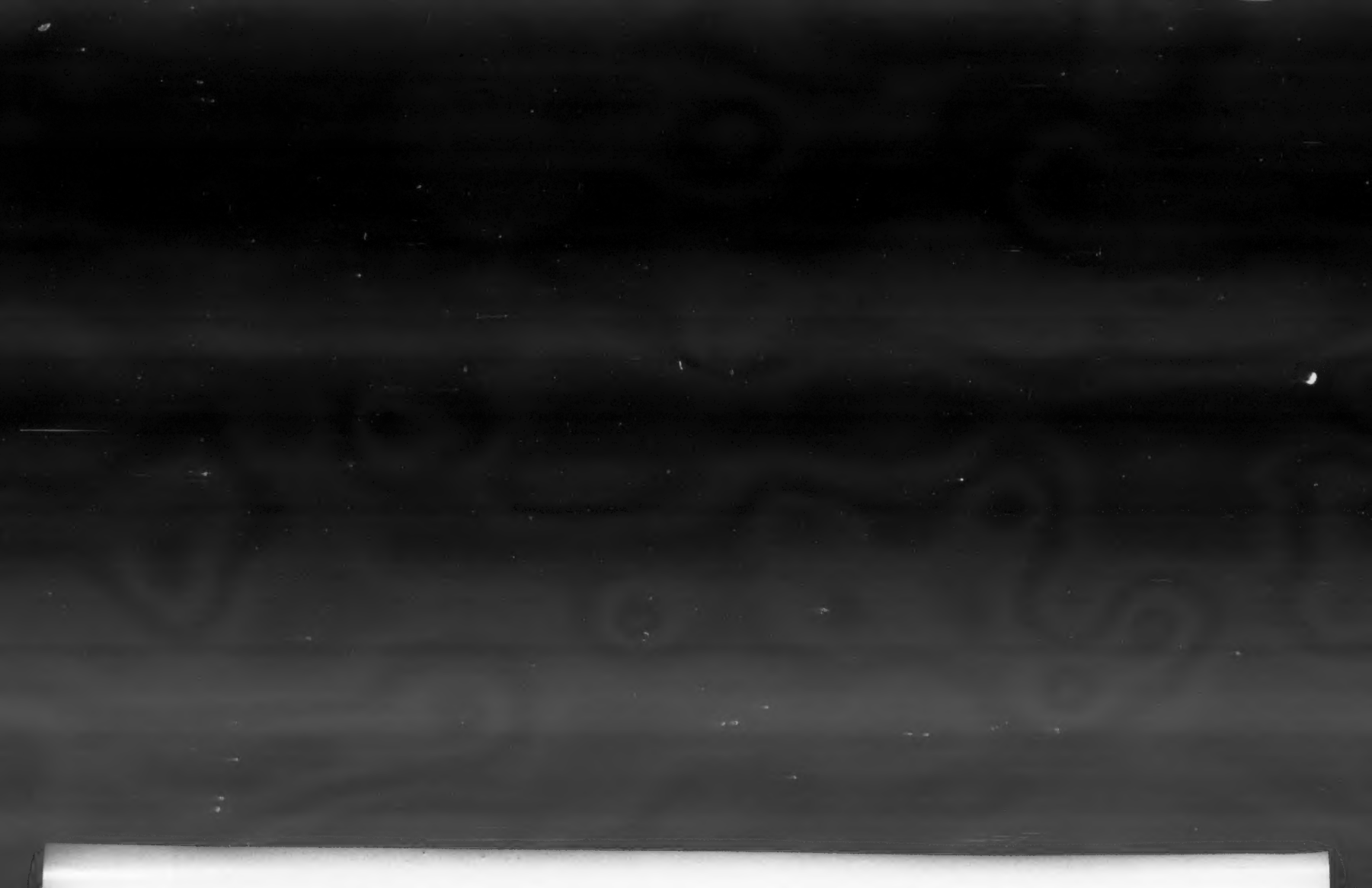
PLATE 178

FIG. 17. Roentgenogram of neurocytoma with calcification in the occipital region.

FIG. 18. Roentgenogram of calcified oligodendroglioma of the frontal region.

FIG. 19. Roentgenogram of calcified sphenoid ridge meningioma.

FIG. 20. Roentgenogram of calcified meningioma of the olfactory groove.



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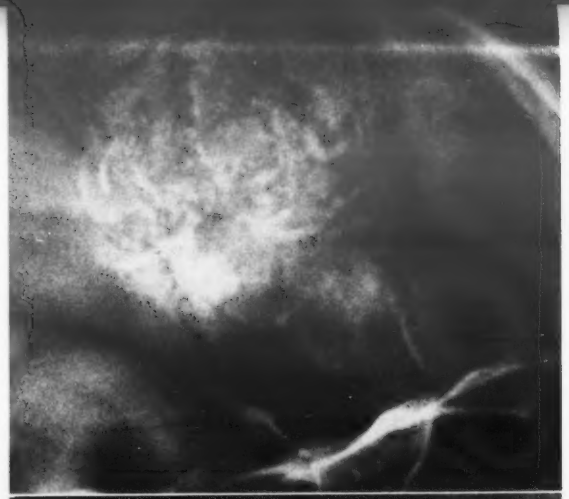

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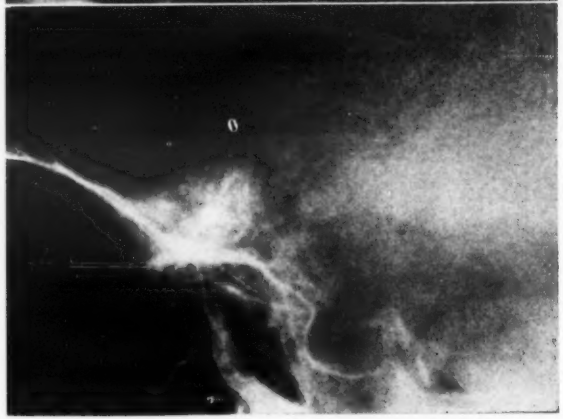

PLATE 179

FIG. 21. Roentgenogram of a calcified intrasellar craniopharyngioma.

FIG. 22. Roentgenogram of a calcified intrasellar and extrasellar craniopharyngioma.

FIG. 23. Roentgenogram of calcification in a chromophobe adenoma of the pituitary gland.

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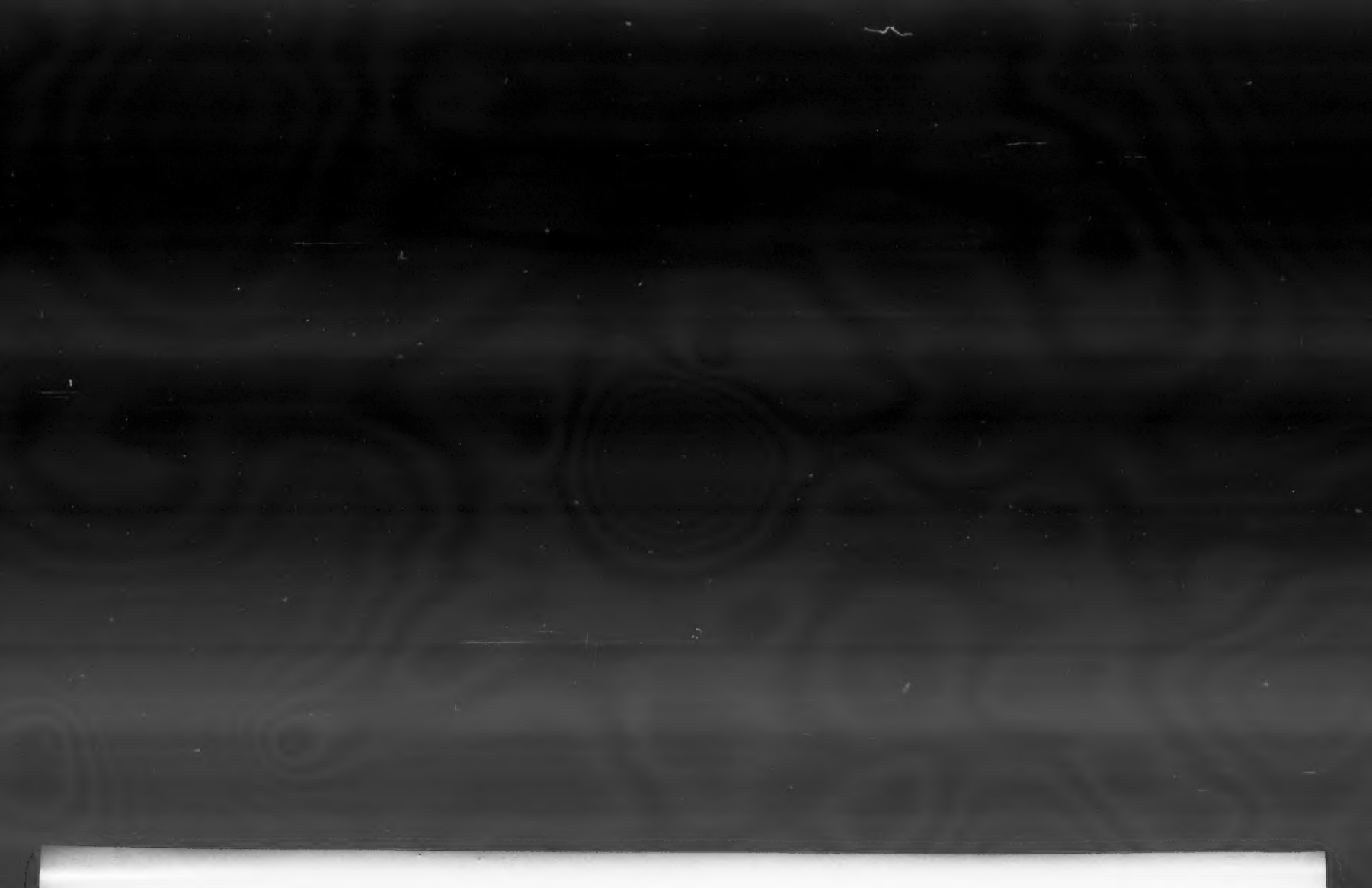
Martin and Lemmen Calcification in Intracranial Neoplasms

PLATE 180

FIG. 24. Ventriculogram showing calcification in cerebellar astrocytoma of the posterior fossa.

FIG. 25. Ventriculogram showing calcification in ependymoma of the posterior fossa.



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A HISTOCHEMICAL STUDY OF IRRADIATED BONE*

M. S. Burstone, D.D.S.†

(From the University of Illinois, College of Dentistry, Chicago 12, Ill.)

The pathogenesis of irradiation changes in the mandibular joint and long bones has been described in some detail.[1,2] Continuous irradiation of developing bone from assimilated radioactive material results in an abnormal reparative process during which there occurs an accentuation of the processes of bone formation as well as resorption. Following internal administrations of beta emitters‡ (P^{32} or Sr^{89}) bone exhibits certain characteristic changes. These include replacement of hematopoietic cells by "gelatinous" marrow containing abnormal reticular cells. Subsequently, atypical bone develops in the marrow, especially near the damaged epiphyseal cartilage. Furthermore, large numbers of osteoclasts may be seen adjacent to these spicules of bone.

In an attempt to elucidate the pathogenesis of irradiation changes as suggested by the earlier work, certain histochemical methods were employed in order to study the bone matrix and related intracellular precursors. Specifically, the periodic acid-Schiff method developed by Hotchkiss and others for demonstration of carbohydrates and glycoproteins was utilized.[3,4] This made possible a study of carbohydrate-protein complexes in the bone matrix as well as within cells.

Methods

Ten C57 mice, 1 to 3 days old, were injected subcutaneously with a carrier-free solution containing radioactive phosphorus, P^{32}, as sodium phosphate. The dosage was approximately 20 μc. per gm. of body weight. Controls consisted of uninjected immature mice of the same age as well as mice injected with non-radioactive phosphate solution. Another series of 8 3-day old mice were injected adjacent to the knee joint with a suspension of chromic phosphate containing P^{32} (activity, 10 microcuries per cc. of suspension). The opposite knee joint was also studied as a control. Other controls for this series were injected in the same area with a non-radioactive suspension of chromic phosphate.

Seven days following injection all experimental and control mice

* This work was aided in part by Grant C-1373 (C) from the National Cancer Institute, U.S. Public Health Service, and by Grant DA-V9-007-MD17 from the Surgeon General's Office, Department of the Army.

Received for publication, June 16, 1952.

† Now at University of Chicago, Department of Anatomy, Chicago 37, Ill.

‡ The P^{32} and radioactive chromic phosphate were supplied by Abbott Laboratories, Chicago, on allocation from the Isotopes Division, U.S. Atomic Energy Commission.

were sacrificed. The knee joints were dissected and the tissues frozen and dried using the Altmann-Gersh technic.[5] All tissues were frozen in isopentane chilled in liquid nitrogen (approximately −150° C.) and then were dehydrated in a vacuum (approximately −30° C.). The undecalcified specimens were then infiltrated *in vacuo* with paraffin (melting point, 60° to 62° C.), embedded, and sectioned at 6 μ. After mounting on albuminized slides, some sections were deparaffinized in xylol, denatured overnight in absolute alcohol, and stained for polysaccharide-protein complexes using the periodic acid-Schiff method.

This periodic acid-Schiff method involves the oxidation of specific carbohydrate-hydroxyl groups to aldehydes. These aldehydes in turn react with leukofuchsin to produce a red color, thus indicating the localization of carbohydrate-containing substances.

Since the monosaccharides, polysaccharides, glycogen, free aldehydes, and protein-lipid complexes are stained, various procedures were followed in order to differentiate these periodic acid-Schiff positive compounds. Various serial sections of each specimen were treated in the following manner: Simple sugars were dissolved by water (M/15 phosphate buffer pH 7). Glycogen, which stains intensely, was removed by incubation with a mixture of alpha and beta amylase (1 mg./cc. pH 6 phosphate buffer M/15). Free aldehydes were stained without the use of an oxidant, periodic acid, while protein-lipid complexes were removed by treatment of the specimen with methanol-chloroform before staining. Intracellular and extracellular red-stained material which remained following these solubility and enzyme tests is believed to constitute a carbohydrate-protein complex oriented as a polymer.

A group of specimens deparaffinized in petroleum ether were treated with enzymes which have been demonstrated to alter glycoproteins, and then were stained using the periodic acid-Schiff method. Undenatured specimens were incubated with trypsin (1/10 mg./cc. in pH 7 buffer M/15). Another group of undenatured sections was treated with hyaluronidase (1350 turbidity reducing units per cc. dissolved in pH 6 phosphate buffer M/15) for 15 and 30 minutes at 37° C., and then stained according to the Hotchkiss method.[3] Still other undenatured sections were treated with collagenase* (1 mg./cc. in Palitzsch's buffer, pH 7)[6] for 1, 6, and 12 hours, and stained for glycoprotein.

Control slides for the aforementioned enzyme tests were incubated in the buffer lacking the enzyme.

A series of undenatured sections was studied for the presence of

* Type A collagenase (B612F) was kindly furnished by the Wellcome Physiological Research Laboratories, Beckenham, England.

alkaline phosphatase by the use of a modification of the Gomori method.[7] Controls for this series were treated with a reagent which lacked sodium glycerophosphate.

The presence of total phosphate-carbonate, presumably as a calcium salt, both intracellularly and extracellularly, was studied by the use of the histochemical modification of the von Kossa method devised by Gersh.[8] In this method, tissue sections are treated with a concentrated silver nitrate reagent and then exposed to ultraviolet light. Areas which contain phosphate-carbonate appear as granules of reduced silver.

For the purposes of orientation as well as morphologic studies, some Hotchkiss-stained material was counterstained with malachite green (25 mg. per cent solution). In addition, other sections were stained with hematoxylin and eosin.

Observations

Control Mice

The epiphyseal cartilage and adjacent bones (tibia and femur) of animals injected with non-radioactive solutions and suspensions appeared histologically identical to untreated controls in all staining reactions. Occasionally, however, macrophages were seen in the area of inert chromic phosphate.

Mice Receiving Systemic P^{32}

Seven days following injection of soluble phosphate containing P^{32}, the marrow cells of the large marrow cavity were replaced by atypical reticular cells. Stained with hematoxylin and eosin, they appeared as enlarged spindle cells with atypical nuclei and long cytoplasmic processes (Figs. 1 and 3). Few normal osteoblasts were present.

Many groups of these cells were embedded in a matrix which stained lightly with eosin. This matrix contained patches of immature bone. Multinucleated cells (presumably osteoclasts) were closely associated with both old and new bone. In many areas, the bone matrix adjacent to the osteoclasts appeared irregular, and basophilic granular material was present.

With the periodic acid-Schiff method the atypical reticular cells, osteoclasts, and the bone stained more intensely than the reticular cells and bone matrix of the controls (Figs. 2 and 4). Moreover, the intercellular matrix associated with the altered marrow also stained pink or red.

The bone matrix adjacent to single and multinucleated cells (osteoclasts) stained intensely and appeared as a dark red band. Granules and also globules of red-staining material were present in and adjacent

to the irregularly outlined bone spicules, giving the appearance of a dissolution of the matrix (Figs. 5 and 6).

Following incubation with amylase, large amounts of Hotchkiss-positive granular material (glycogen) were removed from the cytoplasm of reticular cells and osteoclasts, which essentially constituted the irradiated marrow. Nevertheless, both reticular cells and bone matrix still stained more intensely than normal controls.

Incubation of both control and experimental material with trypsin, hyaluronidase, and collagenase resulted in no appreciable change in the stainability of Hotchkiss-positive material.

The marrow of experimental material treated to reveal the presence of alkaline phosphatase showed a striking aggregation of dark granules in the cytoplasm of the atypical reticular cells, indicating the presence of phosphatase in the cytoplasm. The marrow of normal controls exhibited a minimal localization of phosphatase in the reticular and other cells as contrasted to the irradiated material.

With the modification of the von Kossa method, the irregular spicules of immature bone in the irradiated marrow contained densely packed masses of silver granules, indicating the presence of phosphate-carbonate. The latter was present also in the atypical reticular cells as well as in areas of immature bone which formed in the marrow spaces. Normal bone matrix gives a positive von Kossa reaction but the marrow cells do not contain granular material.

Mice Receiving Local P^{32} as Chromic Phosphate

Study of knee joints irradiated locally by injection of chromic phosphate adjacent to the periosteum revealed changes qualitatively similar to those described for mice receiving soluble P^{32} solution. The knee joint opposite the injected joint appeared similar to the knee joints of control mice.

Discussion

Radioactive phosphorus, when administered systemically, or in a local site adjacent to bone, produces a continuous irradiation of the bone marrow. There result a destruction of normal marrow and its replacement by abnormal connective tissue (reticular) cells. These cells are characterized by increased amounts of glycogen, glycoprotein, alkaline phosphatase, and phosphate-carbonate presumably present as calcium salt. They are associated with an amorphous glycoprotein matrix, which may become calcified to form immature bone.

It is believed that under normal as well as pathologic conditions, cells of connective tissue origin, including osteoblasts, elaborate the

glycoprotein ground substance, or its precursor.[9] However, the glycoprotein matrix laid down following irradiation is conspicuously different from normal matrix seen in animals of comparable age. It stains more intensely with the periodic acid-Schiff method, applied under standard conditions, and an appreciable fraction is water soluble. By analogy with the work of Gersh and Catchpole,[9] it may be postulated that this matrix represents glycoprotein in a lower state of aggregation or polymerization than normal. Such a state of the matrix, *per se,* is indicated by the work of Cobb[10] and Heller[11] to favor calcification, a transformation agreeing with the present findings.

It may be further supposed that the secretion of this altered ground substance is part of a reparative process mediated by the abnormal reticular cells of irradiated marrow. The enhanced amounts of glycogen and alkaline phosphatase present in these cells would support this. Alkaline phosphatase has been related to the formation of calcified protein matrices,[10,12] and glycogen in the cells of regenerating epithelium, for example, has been held to furnish a source of energy for protein synthesis.[13] In irradiated marrow, the active proliferation and secretion of abnormal reticular cells may be interpreted as a repair process utilizing cellular glycogen for energy.

Appearances suggesting matrix dissolution are present also. It is highly unlikely that these would be direct effects of irradiation, since extremely high dosages are usually required to produce breakdown of organic substances. Matrix breakdown is closely associated with cellular activity, and intensely stained areas and granules are observed adjacent to osteoclasts. Dissolution would seem, therefore, to be closely related to the secretion of mucolytic or depolymerizing enzymes by these cells.

Conclusions

Radioactive phosphorus administered systemically or locally (adjacent to bone) results in a destruction of the normal bone marrow and subsequent replacement by abnormal reticular cells.

The abnormal reticular cells contain greater than normal amounts of glycoprotein, glycogen, and alkaline phosphatase, and are associated with a glycoprotein-containing matrix which calcifies.

I wish to thank Dr. M. B. Engel for his counsel, and Miss Edith Crook for technical assistance.

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DESCRIPTION OF PLATES

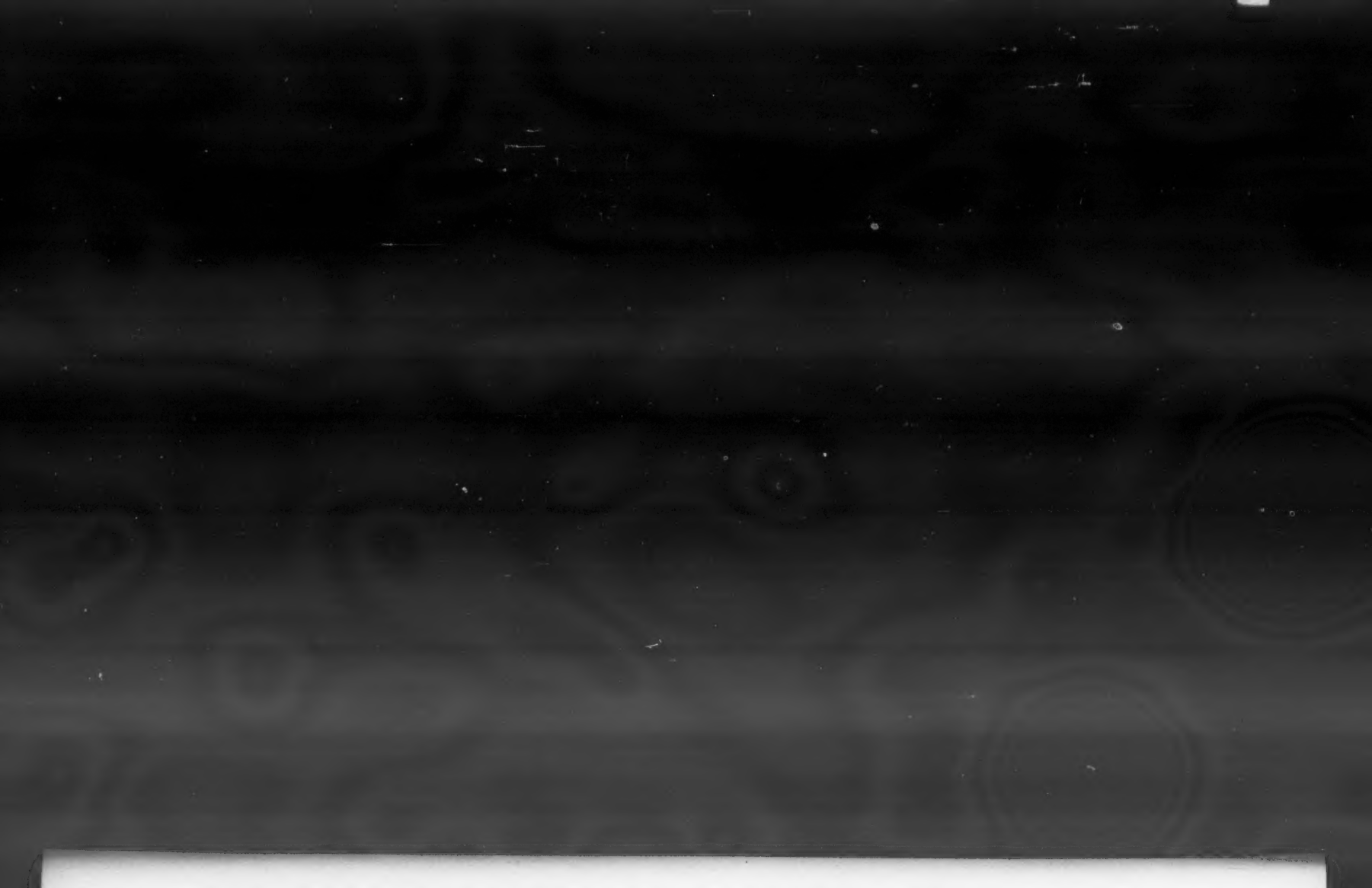
PLATE 181

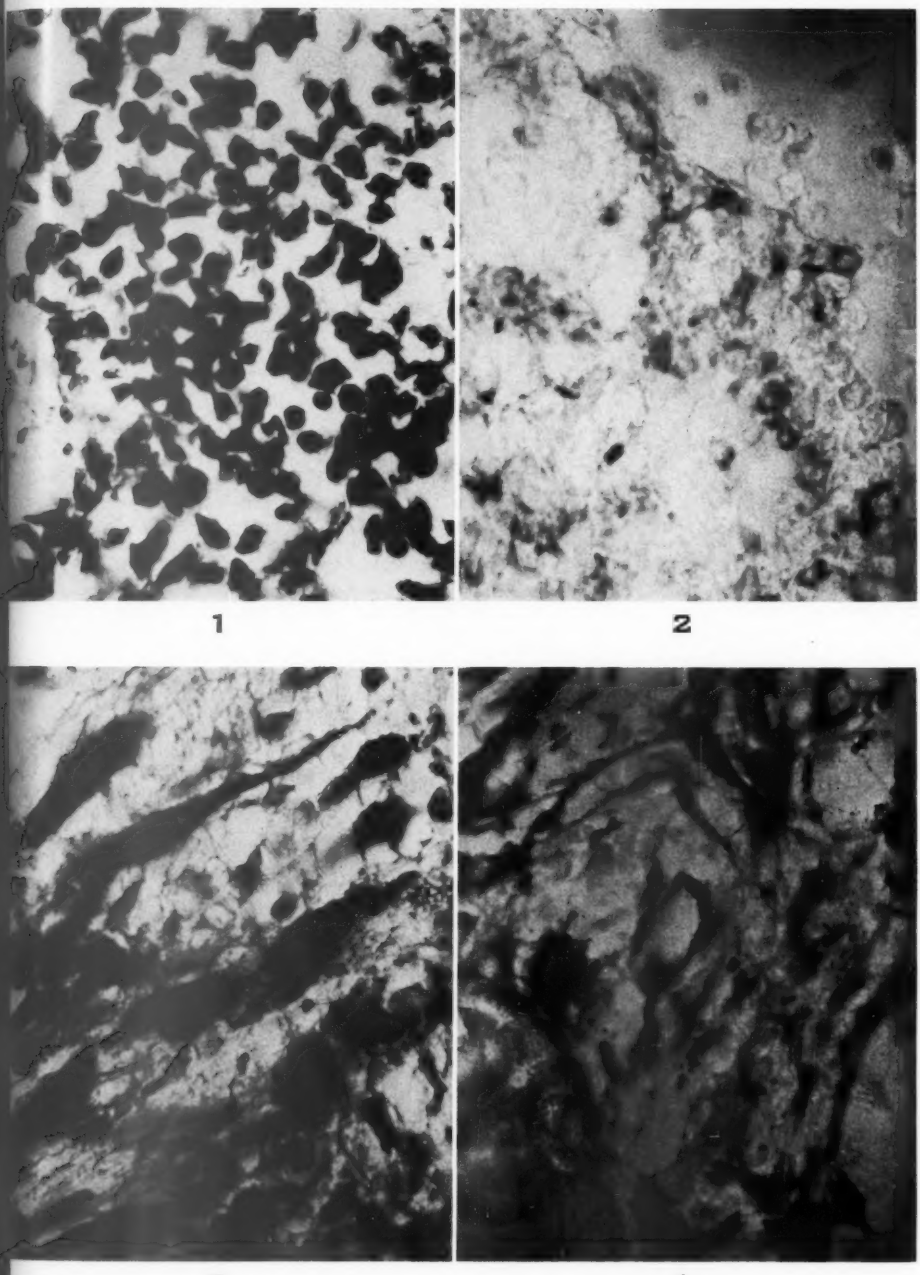
FIG. 1. Normal marrow of tibia, frozen-dried, undecalcified, and stained with hematoxylin and eosin. × 945.

FIG. 2. Normal marrow of tibia, frozen-dried, undecalcified, and stained using the periodic acid-Schiff method. Relatively little periodic acid-Schiff positive material is present. × 945.

FIG. 3. Irradiated tibia showing atypical reticular cells which have replaced the normal marrow. Hematoxylin and eosin. × 945.

FIG. 4. Irradiated tibial marrow stained using the periodic acid-Schiff method. The dark-staining areas represent intracellular glycogen and glycoprotein. × 945.





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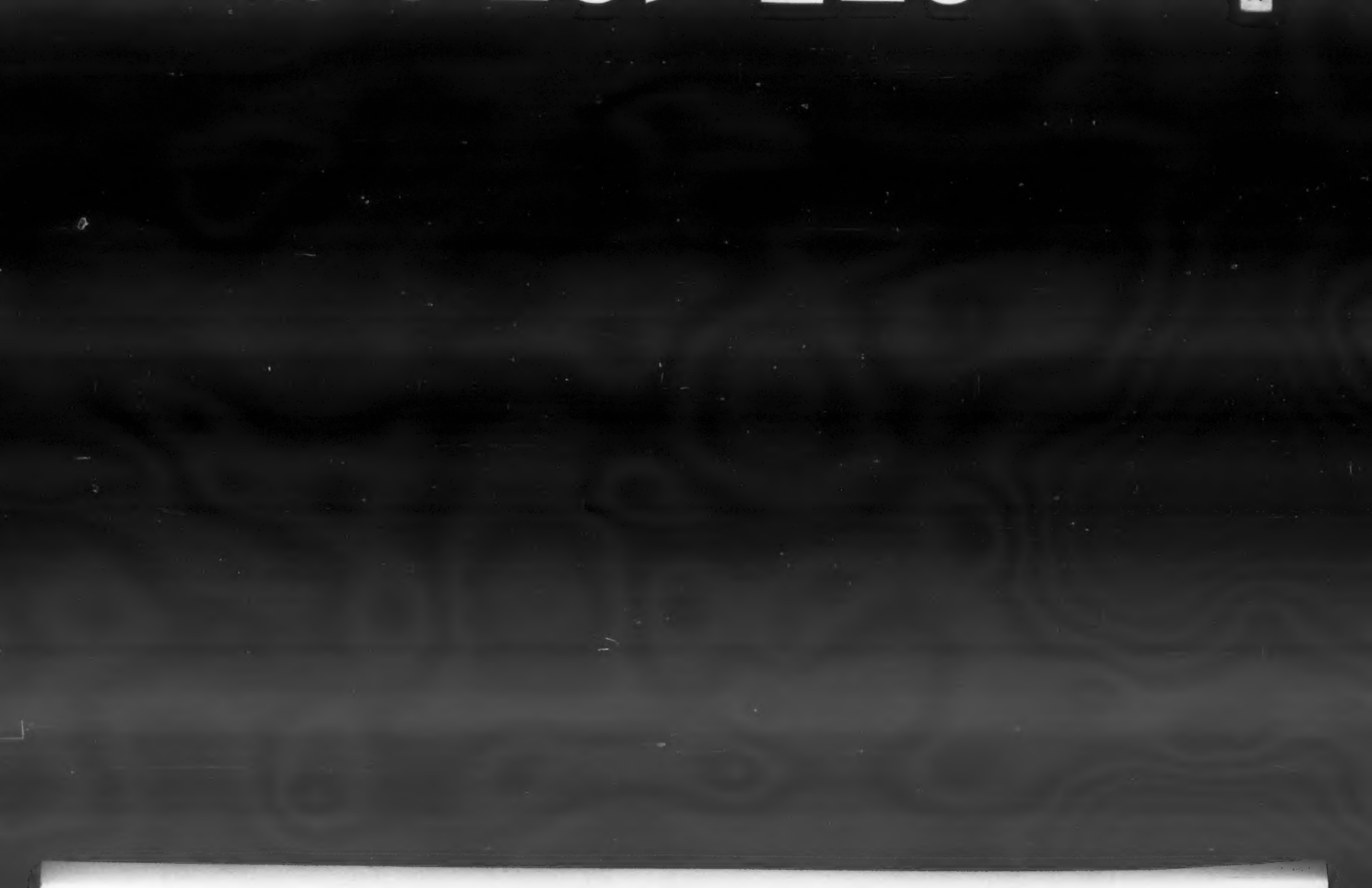
Burstone Histochemical Study of Irradiated Bone

PLATE 182

FIG. 5. Area of frozen-dried, undecalcified epiphyseal bone from a normal control animal, stained by the periodic acid-Schiff method. × 1200.

FIG. 6. Area of frozen-dried, undecalcified epiphyseal bone from an irradiated animal stained by the periodic acid-Schiff method. The staining reaction is less homogeneous than seen in the normal controls, and dark granular appearing areas associated with bone resorption are present. × 1200.



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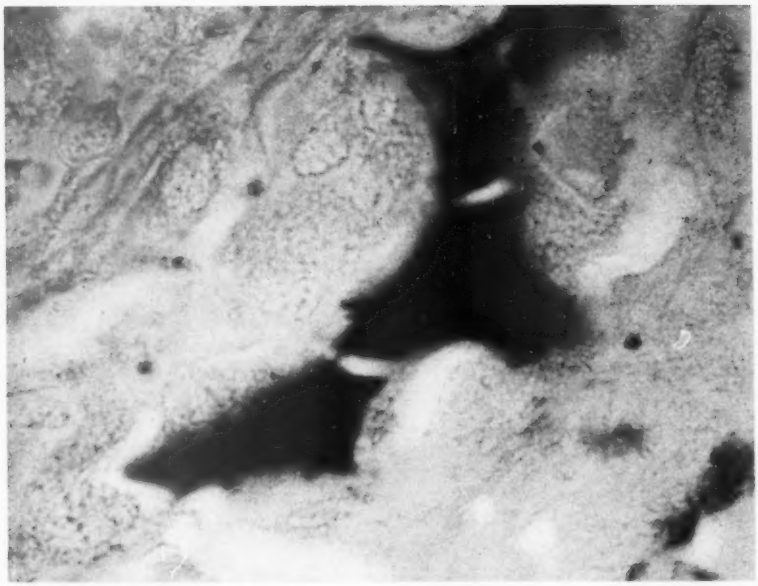

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THE EFFECT OF ESTROGENS ON THE GROWTH APPARATUS OF THE BONES OF IMMATURE RATS *

ANN M. BUDY, B.S., MARSHALL R. URIST, M.D.,† and FRANKLIN C. MCLEAN, M.D.
(From the Department of Physiology, Division of Biological Sciences, University of Chicago, Chicago 37, Ill.)

The laboratory rat displays a peculiar reaction to certain steroid substances which have estrogenic action. Young growing rats, but not guinea-pigs, hamsters, rabbits, cats, or dogs, exhibit an increase in the length and density of the metaphyses of the long bones in response to moderate doses of estrogenic substances.[1,2] Grossly, and in roentgenograms, these changes are easily confused with the endosteal formation of new bone observed in birds[3] and mice[4] treated with estrogen, but the microscopic appearances in the rat are quite different. That these differences in the reaction of the bone to estrogens exist was shown by Day and Follis,[5] who correctly interpreted the effects of estrogens on immature rats as the result of an inhibition of resorption, although certain later writers[6,7] have put a different interpretation on their observations.

Ross and McLean[8] adopted the term "growth apparatus" for the mechanism by which growth occurs in the long bones of rats. This paper will show that the action of moderate doses of estrogens upon the bones of rats is confined to a specific effect upon one particular part of the growth apparatus. This effect does not interfere with the processes which lead to the increase in length of the long bones, but it does affect a process involved in the readjustment of the architecture of the bones to the increase in length, *i.e.*, the resorption of the spongy bone in the metaphysis. The specificity of this effect in rats excludes the endosteal formation of new bone as observed in birds and in mice, and excludes any effect of estrogens on intramembranous bone formation, or on the skeleton of mature rats, since the growth apparatus functions only in relation to endochondral growth of bone.

MATERIALS AND METHODS

Rats of the Wistar, Sprague-Dawley, or hybrids of the two strains, fed an *ad libitum* diet of lab chow checkers were used in all of the experiments. Unless otherwise stated, the rats were 26 to 30 days old,

* These investigations were aided by grants from the National Institutes of Health, U.S. Public Health Service; from the Josiah Macy, Jr. Foundation; and from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Received for publication, June 30, 1952.

† Now at Wadsworth Hospital, Veterans Administration Medical Center, Los Angeles, Calif.

and weighed from 50 to 70 gm. Histologic sections of the bones were made by technics reported by McLean and Bloom.[9] Roentgenographic studies were made on all rats either at frequent intervals throughout the experiment or at necropsy. The steroid nomenclature as interpreted by Mason[10] and later by Fieser and Fieser[11] will be employed in this paper. Estradiol benzoate in this report refers to the formerly designated "α"–estradiol benzoate, and will be described as 17 β–estradiol benzoate only where the two C_{17}-epimers are compared in the experimental procedures. Formerly designated "β"–estradiol benzoate will be described as 17 α–estradiol benzoate throughout the paper.

Effect of Estrogens on Endochondral Ossification

Methods

Thirty-two immature rats were injected with estradiol benzoate in corn oil in doses of 1.0, 2.0, or 4.0 mg. weekly over a period of 1, 2, 4, or 8 weeks. One oil-injected control and 2 experimental rats were necropsied at 2, 3, 5, and 9 weeks. The entire skeleton was examined by means of roentgenograms, and the right tibiae were prepared for histologic examination. These methods of study were used routinely in all experiments unless otherwise stated.

Thirteen immature rats were injected with 0.1 mg. of 17 α–ethinylestradiol in saline solution every other day over a period of 8 weeks. Four rats of similar age and range of weights were injected with saline solution and used as controls. One control rat and 3 estrogen-treated rats were sacrificed at 1, 2, 4, and 8 weeks of the experiment.

Six immature rats (aged 21 days) were injected with diethylstilbestrol in doses of 2.0 mg. weekly over a period of 12 weeks. One control and 2 experimental rats were sacrificed at 4, 8, and 12 weeks.

Results

Moderate to large doses of estrogen, varying from 1.0 to 4.0 mg. per week, produced histologic changes in the skeletons of young growing rats, different from those seen in the bones of similarly treated mice. There was no endosteal bone formation, but there were striking effects upon the process of endochondral ossification. With large doses (4.0 mg. weekly), the first changes observed were at the upper end of the tibia as early as 9 days. At 15 days the spongiosa was twice the normal length. After this period the spongiosa further increased in length, and increased density of the metaphysis could be observed even in the gross specimen. Normally, in the epiphyseal lines of the rat, the matrix of about 5 of 7 cartilage-cell rows is absorbed (Fig. 1),

and new bone is laid down on the remaining spicules of cartilage matrix. In the estrogen-treated rats, the process of resorption of the cartilage matrix was inhibited by moderate doses (3.0 mg. in 3 weeks) to the extent that the matrix of only about 3 of 7 row interspaces was removed. Moderately large doses (2.0 mg. and more weekly) interfered with the cartilage resorption mechanism to such a degree that none of the matrix in the row interspaces was removed (Figs. 2, 3, and 4). The resulting histologic picture was that the spongiosa was elongated and increased in density, and all the bony trabeculae contained cores of deeply staining cartilage matrix. The increase in length of the spongiosa was determined by the duration of treatment or the period of growth under the influence of estrogen. With large doses the growth of the long bones was greatly retarded and the metaphyses were moderately elongated. With moderate doses, the long bones were only slightly shorter than normal, but the metaphyses were greatly elongated, in some instances as much as four times, within a period of 8 weeks. The increase in density of the spongiosa within a specified period was directly proportional to the size of the dose. The metaphysis became so dense that it was indistinguishable from compact bone in the roentgenograms. The persistence of all of the cores of cartilage matrix that are formed from the epiphyseal plate demonstrates conclusively that the effect of estrogens in the rat is primarily upon the process of resorption (Figs. 1, 2, 3, and 4).

The Use of the Rat Tibia as a Method of Measuring the Skeletal Reaction

It was observed that the effects of estrogens on endochondral ossification were most prominent and occurred earlier in the tibial metaphyses than in any of the other long bones. Studies of the density and the length of the metaphyses have shown that the histologic and roentgeongraphic observations could be combined and used quantitatively with accurate results. The density was determined by counting the number of bony trabeculae at the epiphyseal line. The length was determined by comparison with the normal spongiosa and was expressed by symbols of multiples of N (N = normal). The multiples of N increased with a 2 mm. elongation of the spongiosa; an increase in the length of the spongiosa up to 4 mm. was designated as $2 \times N$, an increase up to 4 to 6 mm. as $3 \times N$, an increase up to 6 to 8 mm. as $4 \times N$, and an increase up to more than 8 mm. as $5 \times N$ (Figs. 1, 2, 3, 4, and 5, 6, 7, 8).

This technic was accurate and quantitative results could be repeated

consistently when the rats were carefully selected and the test conditions were uniform with respect to age, dose, and the estrogen. An immature rat of either sex, approximately 30 days old, weaned to a diet of lab chow checkers was considered the standard rat in these experiments. The reaction of the tibial metaphyses from a weekly dose of 1.0 mg. of estradiol benzoate was used as a base line for all measurements.

The Influence of Growth, Age, and Diet

Methods

Two grouped-litters of 8 rats each were injected weekly with estradiol benzoate beginning on the day of birth and then at 7-day intervals with graduated doses of 0.05 mg. until 14 days, of 0.1 mg. until 28 days, and of 0.5 mg. after 35 days. Another litter, injected with olive oil, was used for controls. One rat from each group was sacrificed every 5 days between 5 and 40 days of age.

Three young adult rats (5 to 6 months of age) were injected with a total of 8.0 mg. of estradiol benzoate over 8 weeks. A similar rat was injected with olive oil and was used as a control.

Twelve adult rats, males and females, obtained from the Wistar breeder stock, 9 to 11 months of age, were injected daily with 1.0 mg. of estradiol benzoate in corn oil. One control and 2 experimental rats were sacrificed after 1 week of treatment and the remaining control and estrogen-treated rats were sacrificed after 4 weeks of continuous administration.

Nine weanling rats, weighing from 40 to 50 gm., were divided into two groups and placed on a rachitogenic diet (Steenbock-Black no. 2965) at 21 days of age. One group of 4 rats was given intramuscular injections of 0.1 mg. of 17 α–ethinylestradiol in saline solution every third day for 3 weeks. The second group was given daily injections of 0.5 mg. for 5 days after a period of 3 weeks on the diet. A rachitic control rat in each group received saline injections over the same period of time.

Results

Compared with the newborn mouse, the newborn rat appears to be more resistant to the skeletal effects of estrogen. As will appear, weanling rats exhibit a reaction if estrogen is given as early as the 13th day of age (Table II). However, there is no effect upon resorption before 21 days of age when estradiol benzoate is given on the day of birth and then continued in moderate stimulating doses. Appearance of ossification centers was not accelerated in rats 5 and 10 days

of age. Estradiol benzoate administered to young adult rats had no effect upon bone formation. Mature rats under influence of estrogen showed none of the changes demonstrated by similarly treated immature rats. 17 α–ethinylestradiol given in small doses over a period of 4 weeks will produce a 2 × N reaction in immature rats maintained on an adequate diet. No effect was observed in rats made rachitic and maintained under the influence of estrogen. No anti-rachitic properties could be demonstrated, since the same amount of osteoid tissue was present in the estrogen-treated rachitic rats as in the rachitic controls.

Process of Recuperation

Methods

Thirty-two immature rats were injected with 17 β–estradiol benzoate in corn oil in doses of 1.0, 2.0, or 4.0 mg. weekly over a period of 1, 2, 4, or 8 weeks. An oil-injected control was used for each dose-group. The treatment was discontinued after each period and the rats were allowed to recuperate and grow until the ninth or tenth week after the initial dose. Roentgenograms were taken at necropsy.

Six immature rats were injected with 0.2 mg. daily over a period of 5 days. Three control rats were injected with olive oil over the same period of time. All rats were then allowed to grow for 20 weeks. Roentgenograms were made at 2-week intervals.

Five immature rats were injected with a single dose of 4.0 mg. of estrogen and one control rat was injected with a single dose of olive oil. All rats were allowed to recover over a period of 16 weeks. Roentgenograms were made at weekly intervals until the effects of the estrogen could no longer be demonstrated (Figs. 9, 10, and 11). Seven weanling rats weighing 23 to 27 gm. were given the same dose at 13 days of age and were observed in a similar manner. In this group each rat was its own control.

Five immature rats were injected at 21 days of age with 2.5 mg. of re-crystallized 17 α–estradiol benzoate* over a period of 2½ weeks and were allowed to recover for a period of 13 weeks after treatment was discontinued. Roentgenograms were made at 2-week intervals using each rat as its own control. The animals were sacrificed after 1, 4, and 13 weeks of recovery.

Two litters of 7 newborn rats each were injected with 0.005 mg. of 17 α–ethinylestradiol or 0.5 mg. of diethylstilbestrol every other day

* Crude 17 α-estradiol benzoate was re-crystallized by Dr. Konrad E. Bloch of the Department of Biochemistry. The product obtained had a melting point of 152-153° C. and contained less than 1 per cent of the original 17 β-estradiol benzoate.

over a period of 4 weeks from the day of birth. Another litter was injected with olive oil and was used as controls. Rats from each litter were sacrificed at 2, 4, 6, 12, 14, and 16 weeks of age.

Results

Analysis of the process of recovery from the effects of estrogens on the growth apparatus in the long bones of rats is complicated by two factors. First, the effects of estrogens continue for some time after administration has been discontinued, especially if the hormone is given intramuscularly in oil. Consequently, during the first weeks after treatment is stopped the spongiosa may continue to increase in length, rather than to show signs of recuperation. Second, restoration of the normal cycle of growth and resorption begins, not at the ends of the dense spicules of bone that make up the metaphysis, but at the junction of the epiphyseal cartilage with the spongiosa. The earliest sign of healing, therefore, is a thinning out of the trabeculae in the primary spongiosa. At this stage the dense metaphyseal bone, formed under the influence of estrogen, may appear to be separated from the epiphyseal plate. After some weeks this dense bone is also absorbed, and eventually the normal architecture of the bone is restored.

Table I illustrates experiments undertaken in order to observe the persistence of the effects of estrogens, and the early stages of recovery. Animals were necropsied at the end of the treatment period, which was varied from 1 to 8 weeks. Other rats were similarly treated, and permitted to survive for 1 to 8 weeks after administration of the hormone was discontinued. When 2.0 mg. of estrogen was administered during a period of 1 week, and the animal was permitted to survive for another 8 weeks, there was recovery of the resorption mechanism in the primary spongiosa. All other animals in the series in Table I failed to show recovery.

Table II illustrates the results on animals treated for varying lengths of time, and permitted to survive to complete recovery. Seven weanling rats given single doses of 4.0 mg. of 17 β–estradiol benzoate did not arrive at the maximum length of the spongiosa until 6 weeks had elapsed, and recovery, as observed by roentgenograms, was not complete until after another 6 weeks. When the same dose and the same estrogen was administered to 28-day-old rats, maximum length of the spongiosa was observed at 8 weeks and recovery was not complete until 8 weeks later.

Immature rats treated with re-crystallized 17 α–estradiol benzoate showed an early but slight response in 2 weeks; complete recovery was not attained until 6 weeks later.

Newborn rats treated with 17 α-ethinylestradiol from the day of birth showed a slight increase in the density of the spongiosa in 4 weeks. Two weeks after the estrogen was discontinued, the density of the metaphysis was slightly greater than normal and recovery was rapid and complete within the following 4 weeks. Diethylstilbestrol produced a slightly greater effect upon the metaphysis. The greatest

TABLE I

Comparison of the Effects of Continuous Treatment with That of Treatment and Growth

Dose	Duration of treatment	Group I Necropsied at end of experiment: Reaction of tibia	Group II Necropsied at end of growth period following treatment: Period of growth	Group II: Reaction of tibia
mg.	*weeks*		*weeks*	
1.0	1	N	8	N
2.0	1	<2×N	8	2×N R*
2.0	2	<2×N	7	2×N R†
4.0	1	<2×N	8	2×N R†
4.0	2	<2×N	7	2×N R†
4.0	4	<2×N	5	2×N R†
8.0	2	2×N	7	2×N R†
8.0	4	2×N	5	
8.0	8	<3×N	1	<3×N R†
16.0	4	3×N	5	<3×N R†
16.0	8	3×N	2	<3×N R†
32.0	8		2	<3×N R†

R*, with recovery of resorption mechanism in the primary spongiosa.
R†, no recovery.

TABLE II

Rate of Recovery from Different Estrogens

Estrogen	Total dose	Period of treatment	Age at initial dose	Maximum reaction of tibia	Time of maximum reaction	Time of complete recovery
	mg.	*days*	*days*		*weeks*	*weeks*
17β-estradiol benzoate	1.0	5	27	<2×N	3	12
17β-estradiol benzoate	4.0	Single dose	13	3×N	6	12
17β-estradiol benzoate	4.0	Single dose	28	4×N	8	16
Re-crystallized 17α-estradiol benzoate	2.5	17	21	2×N	2	8
17α-ethinylestradiol	0.07	28	At birth	<2×N	4	8
Diethylstilbestrol	7.0	28	At birth	<2×N	2	8

increase in density was observed in 2 weeks after the hormone was discontinued; a few remaining spicules were observed at 4 weeks, while a few spicules were observed in only one of 3 rats at 6 weeks of recovery.

In these experiments, the ages of the animals at the time of the initial dose is as important a factor as is the dose itself in the progress of recuperation from the effects of estrogens. In general, complete recovery from the effects in bone can be demonstrated in 16 weeks or less provided the dose is moderate and the rat is of weaning age at the beginning of the treatment.

Effects of Other Steroid Hormones and Hormone-Like Substances

Methods

Forty immature rats were divided into three groups. Each group was further divided into four sub-groups and these were treated in the following manner: Each sub-group was injected with olive oil and used as controls, or with 7.0 mg. of testosterone propionate, or with 0.28 mg. of estradiol benzoate, or with the same doses of both hormones over a period of 2 weeks. A second group of rats was similarly treated with the same dose over a period of 4 weeks. A third group was injected with olive oil and used as controls, or with 14.0 mg. of testosterone propionate, or with 0.56 mg. of estradiol benzoate, or with both hormones over a period of 8 weeks. Measurements of the length of the spongiosa were made on histologic sections of the tibiae. The averages of several sections were used on each tibia, and the averages of the animals in each sub-group as well as the individual averages are shown in Table III.

Nine immature rats (21 days of age) were injected with a crude extract of 17 α–estradiol benzoate in olive oil in 1.0 mg. weekly doses over a period of 12 weeks. A second group was injected with weekly doses of 0.5 mg. of re-crystallized 17 α–estradiol benzoate over a period of 8 weeks. Oil-injected controls were necropsied at the termination of each experiment.

Six immature rats (24 days of age) were injected with biweekly doses of 0.5 mg. of 3-methyl bisdehydrodoisynolic acid over a period of 2 weeks. One control and 2 experimental rats were sacrificed at 3, 6, and 12 weeks.

Fourteen immature rats (21 days of age) were divided into three groups. One group was injected with 1.0 mg. of desoxycorticosterone acetate daily for 4 weeks, then 4.0 mg. every other day for 4 weeks. The second group was injected with 4.0 mg. in four doses weekly over 8 weeks, then 8.0 mg. doses weekly for 4 weeks. The third group was given 2.0 mg. weekly for 8 weeks, then five 4.0 mg. doses weekly for 4 weeks. Roentgenograms were made at 2-week intervals. Oil-in-

jected controls and experimental rats were sacrificed at 4, 8, and 12 weeks.

Results

The results following administration of testosterone alone were, in this small series of animals, equivocal. Of the 9 rats given such treatment, 3 males and one female showed an increase in length, density,

TABLE III

The Effects of Testosterone Propionate, Estradiol Benzoate, or Both on the Length of the Spongiosa

Hormone	Number of animals	Total dose	Length of spongiosa	
			Average	Individual
		mg.	mm.	mm.
Two weeks				
Control	2		2.29	1.51; 3.07
Testosterone	2	7.00	2.81	2.87; 2.76
Estradiol benzoate	3	0.28	3.25	3.45; 3.23; 3.07
Testosterone and estradiol benzoate	3	7.00 0.28	2.87	3.03; 2.76; 2.83
Four weeks				
Control	5		1.54	1.21; 1.51; 1.29; 1.65; 2.08
Testosterone	5	7.00	1.95	2.37; 1.00; 2.80; 2.44; 1.15
Estradiol benzoate	4	0.28	3.81	5.03; 3.38; 4.05; 2.80
Testosterone and estradiol benzoate	5	7.00 0.28	3.77	2.44; 4.45; 4.12; 3.86; 4.01
Eight weeks				
Control	2		1.36	1.29; 1.43
Testosterone	2	14.00	2.26	2.37; 2.15
Estradiol benzoate	4	0.56	4.39	2.87; 3.77; 5.22; 5.70
Testosterone and estradiol benzoate	2	14.00 0.56	4.99	5.17; 4.81

and number of osseous spicules in the primary spongiosa. The remaining 5 rats, 4 males and one female, showed no bone changes, nor did the metaphyses increase significantly in length or density. The variations observed were not significant in this small series. Testosterone and estrogen, when given simultaneously every other day for 8 weeks, produced approximately the same effect as with estrogen alone. Testosterone did not repress the action of the estrogen on the epiphyseal cartilage of the rat (Table III).

Immature rats treated with a crude extract of 17 α–estradiol benzoate showed a reaction far more intense than in rats treated with the re-crystallized hormone. This might have been due to the presence of

17 β–estradiol benzoate contained in the crude extract. The re-crystallized substance produced a response similar to that observed with the less potent estrogens.

Immature rats treated with 3-methyl bisdehydrodoisynolic acid showed a reaction similar to that from estradiol benzoate.

TABLE IV

The Effect of Steroid Hormones and Hormone-Like Substances on the Skeleton of Immature Rats

Hormone	Length and density of the metaphysis			
	2 weeks	4 weeks	8 weeks	12 weeks
Doses of 1.0 mg./wk.				
17β-estradiol benzoate	2×N	3×N	4×N	
Crude 17α-estradiol benzoate		2×N	3×N	4×N
Re-crystallized 17α-estradiol benzoate		2×N	2×N	
3-methyl bisdehydrodoisynolic acid	N	<2×N	3×N	4×N
Varying doses				
Diethylstilbestrol, 2.0 mg./wk.	N	<2×N	<2×N	<3×N
17α-ethinylestradiol, 0.35 mg./wk.	N	2×N	2×N	
Testosterone propionate, 3.5 mg./wk.	<2×N	N	N	
1.75 mg./wk.		<2×N	<2×N	
Testosterone, 3.5 mg./wk. plus estradiol benzoate, 0.14 mg./wk.	<2×N	2×N	2×N	
Testosterone, 1.75 mg./wk. plus estradiol benzoate, 0.07 mg./wk.		2×N	2×N	
Desoxycorticosterone, 30.0 mg.		N		
86.0 mg.			N	
152.0 mg.				N

Desoxycorticosterone acetate given in large doses to immature rats did not produce any effect upon endochondral ossification.

Table IV shows the effects of steroid hormones and hormone-like substances upon the skeleton of rats.

DISCUSSION

The results presented herewith demonstrate that the action of estrogens on the bones of rats is confined to a specific effect upon one particular part of the "growth apparatus." This effect is an interference with a process involved in the readjustment of the architecture of the bone to its increase in length, *i.e.*, the resorption of the spongy bone of the metaphysis.

It is well known that, as the bones increase in length by growth at or near the epiphyseal cartilage, the extensions of the bony spicules with cores of cartilage matrix into the marrow cavity are kept thinned out and at approximately constant length by resorption, which keeps pace

with the growth in length. Interference with this resorption, then, unaccompanied by any interference with growth itself, results in an increase in length and density of the spongiosa of the metaphysis. Although the end result superficially resembles the production of endosteal bone induced in birds and in mice by the administration of estrogens, the excess bone is seen to retain the deeply staining cores of cartilage matrix which in the normal course of events should have been resorbed.

It follows, once the mechanism of action of estrogens on the bones of rats is understood, that this action can manifest itself only in the presence of continuing growth, and more specifically growth by endochondral ossification. No similar action upon intramembranous growth of bone is to be expected, and Stahl *et al.*[12] have shown that intramembranous ossification, in the rat, is not affected by estrogens.

Ross and McLean[8] showed that rats which had reached a growth plateau at the age of 6 months, could be induced to produce new metaphyseal bone by the administration of growth-promoting hormone. Day and Follis[5] found that they could not elicit a response to estrogens in rats of a similar age, and this agrees with the observations reported in this paper. These findings are consistent with the thesis of this paper, *i.e.*, that the specific effects of estrogens on bone in rats are limited to the arrest of resorption, and provide further evidence that the estrogens do not have a growth-promoting function.

Further evidence to the same effect is provided by the results of administration of estrogens to rachitic rats, reported in this paper. While growth persists in an abnormal fashion in the rachitic metaphysis, the process of resorption is at a standstill unless healing is under way. Under these circumstances it is not to be expected that estrogens will have any effect upon the rachitic metaphysis, and our findings are in agreement with this. Moreover, although Norman and Mittler[13] reported that estrogens are anti-rachitic in rats, our observations do not confirm this report.

The net result of the action of estrogens on the bones of growing rats is an increase in the length and density of the spongy bone of the metaphysis. But it is misleading to refer to the process itself as "hyperossification," as others have done,[6,7] since this implies an overproduction of bone, rather than the failure to remove excess bone which is the actual mechanism of the process under consideration.

In addition to establishing the character of the mechanism just referred to, certain observations herein reported throw further light on various aspects of the physiologic effects of the estrogens and related substances on the bones of rats, which will be referred to briefly.

Bremer[14,15] reported that antagonistic reactions were observed in rats treated with theelin and testosterone. Under the conditions of our experiments there were no observable differences in the action of estrogens in male and female rats. As to the male hormone, administration of testosterone propionate alone gave equivocal results, in so far as the effects on the growth apparatus were concerned (Table III). More data are needed. Testosterone, when given in combination with estrogen, produced neither an antagonistic nor a synergistic effect. 3-methyl bisdehydrodoisynolic acid was found to be slightly less effective than estradiol benzoate in producing the characteristic failure of resorption of spongy bone in the rat, although it does not lead to endosteal bone formation in the mouse.[4] Desoxycorticosterone did not produce any effect on endochondral ossification, even in extremely large doses.

The effects of different estrogens and estrogen-like substances as shown in Table IV are in agreement with the reports found in the literature.[16-18] The response of the skeleton to the various substances closely follows the metabolism of the estrogen.

There was no acceleration of the appearance of ossification centers in either male or female rats under the conditions of our experiments. This is in agreement with the results of Noback *et al.*[19] Newborn rats treated from the day of birth are more resistant to the skeletal effects of estrogen than are weanling rats. Histologic and roentgenographic observations show that there is no effect upon the resorption before 21 days, when administration is initiated on the day of birth and continued in stimulating doses. Again there is no difference in the response of male and of female rats.

Recovery of the normal mechanism of resorption of metaphyseal spongiosa, after administration of estrogens, occurs rather slowly, especially if large doses are given. Renewal of the resorption process is first seen at the level of the junction of the primary spongiosa and the epiphyseal plate, so that the dense metaphyseal bone, characteristic of the effect of estrogens, becomes separated from the epiphyseal plate by a zone of normal endochondral ossification. Eventually, however, complete recovery occurs, with removal of the excess bone of the metaphysis.

Finally, it should be noted that there is no evidence, from human material, that the effects of estrogens on growing children in any way resemble the effects on rats. The results herein reported are not intended to be extrapolated to man, and there is no justification for doing so. It appears that there are characteristic differences for the species which have been studied,[1] and it is the purpose of this paper to record and interpret the results observed in rats.

Summary and Conclusions

The action of moderate doses of estrogen upon the bones of rats is confined to a specific effect upon one particular part of the growth apparatus, *i.e.*, interference with resorption of the spongy bone in the metaphysis.

The density of the metaphysis and the length of the non-resorbed spongiosa are, within the limits of the tolerance of the animal, directly proportional to the dose and to the period of administration, and are also dependent upon the age of the animal.

Newborn rats, treated with estrogen from the day of birth, show none of these changes until 21 days.

Immature rats show appreciable differences in their response to estrogen. The maximum response is produced when administration of estrogen is initiated when the animal is approximately 30 days old. Younger rats (13 days old) show a much smaller response.

Rats treated with various doses of estrogens recover from the effects with respect to the normal growth of bone.

Testosterone propionate alone did not consistently produce the reactions which followed administration of estrogens. There is neither inhibitory nor synergistic action of testosterone when given with estrogen.

Since the effect of estrogens on the bones of rats is limited to a specific action upon one part of the growth apparatus, which is concerned only with endochondral growth of bone, no similar action upon intramembranous ossification occurs.

Crystalline steroid hormones and hormone-like substances were provided by Dr. Kenneth W. Thompson of Roche Organon, Inc., Dr. E. C. Reifenstein of Ayerst, McKenna & Harrison, Ltd., Dr. Erwin Schwenk of the Chemical Research Division of the Schering Corporation, and Dr. F. E. Houghton of Ciba Pharmaceutical Products, Inc.

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DESCRIPTION OF PLATES 183–186

Photomicrographs of histologic sections of the upper end of tibiae in immature rats treated with estradiol benzoate. The density of the metaphyses and the length of the spongiosa can be determined by comparison with the normal, and the length is expressed by symbols of multiples of N. (N = normal.) Hematoxylin-eosin and azure stain. × 25.

PLATE 183

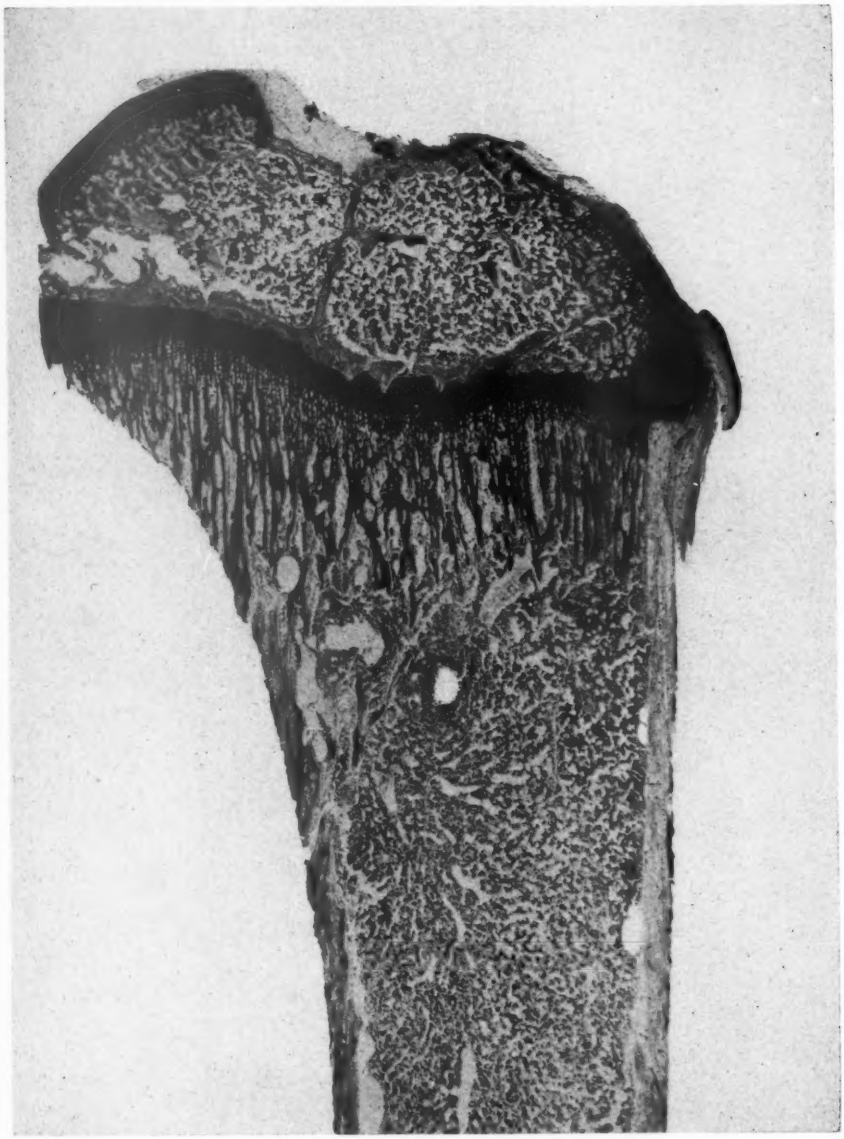
FIG. 1. Control. N is the normal length of the metaphysis.

1

Budy, Urist, and McLean Effect of Estrogens on Growth of Bone

PLATE 184

FIG. 2. Moderately large dose of 2.0 mg., observed after a period of 2 weeks. An increase in the length of the spongiosa up to 4 mm. is designated as 2 × N.

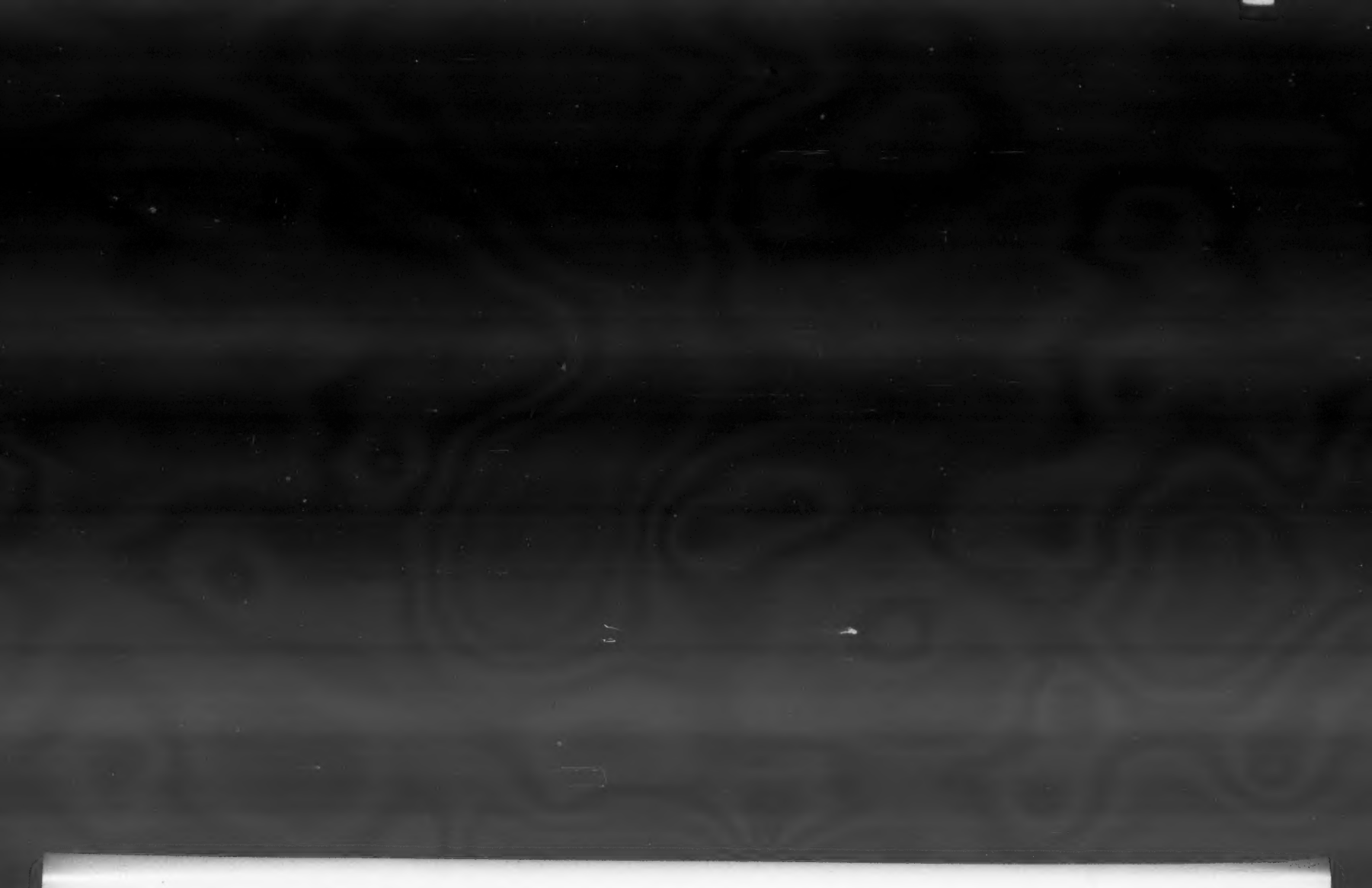


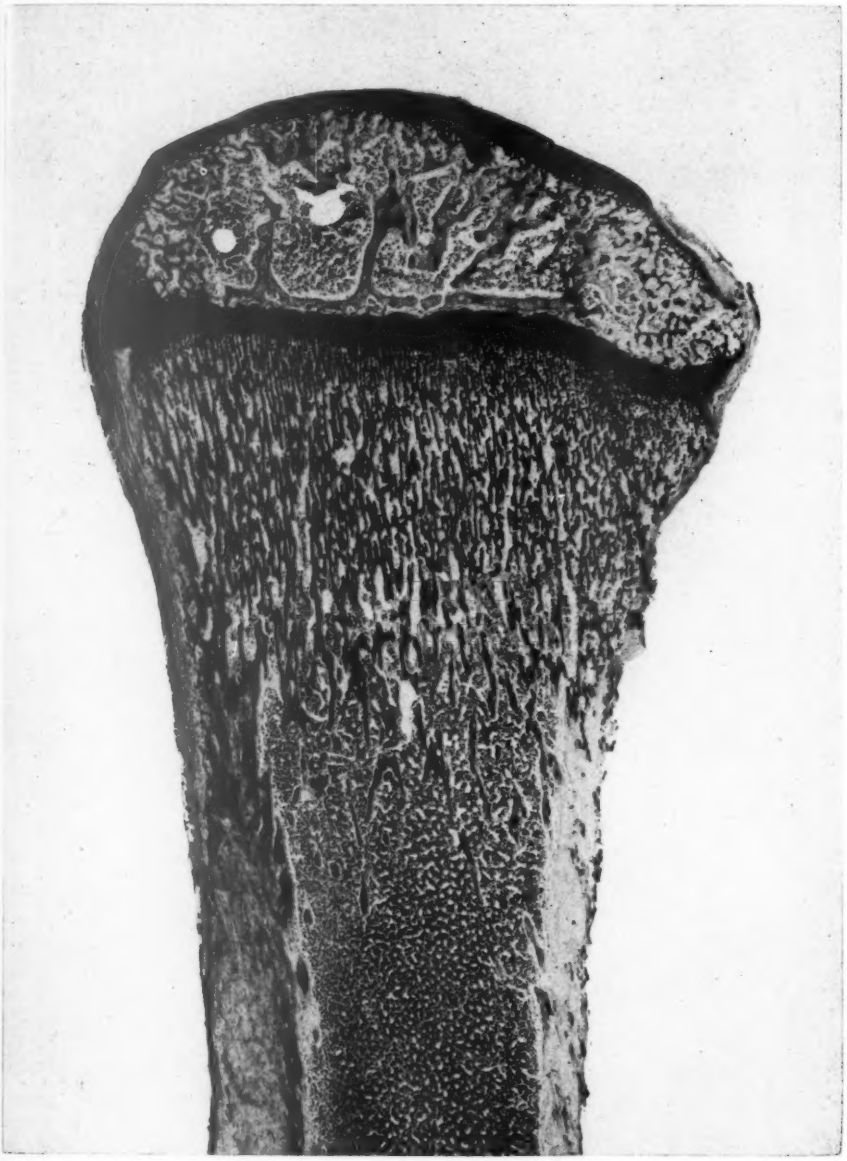
2

Budy, Urist, and McLean Effect of Estrogens on Growth of Bone

PLATE 185

FIG. 3. Large dose of estrogen (8.0 mg.) given over a period of 3 weeks. An increase in the length of the spongiosa to 4 to 6 mm. is designated by 3 × N.



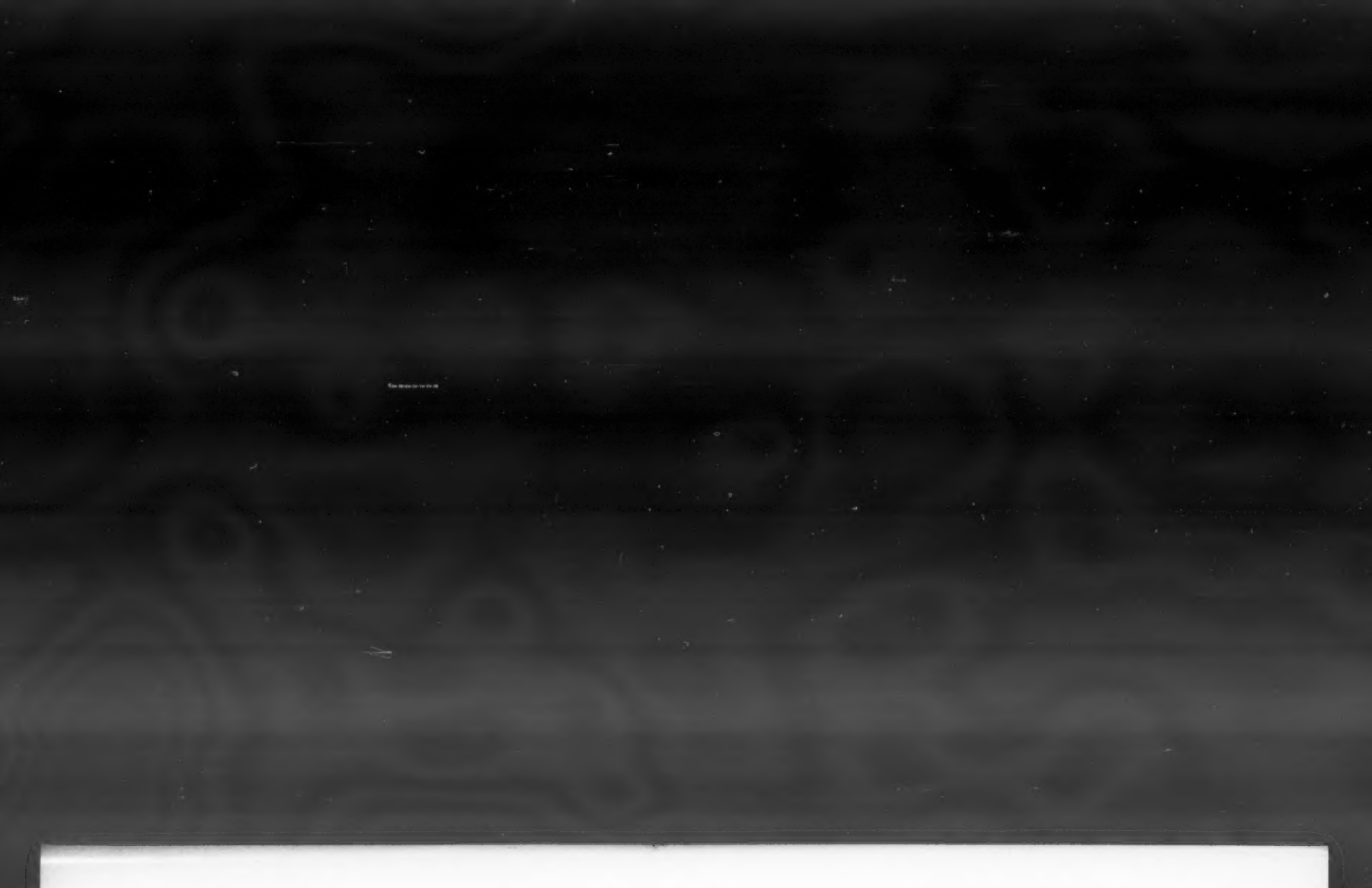


3

Budy, Urist, and McLean Effect of Estrogens on Growth of Bone

PLATE 186

FIG. 4. Large dose of estrogen (8.0 mg.) given over a period of 5 weeks. An increase in the length of the spongiosa up to 6 to 8 mm. is designated by 4 × N.



4

Budy, Urist, and McLean Effect of Estrogens on Growth of Bone

PLATE 187

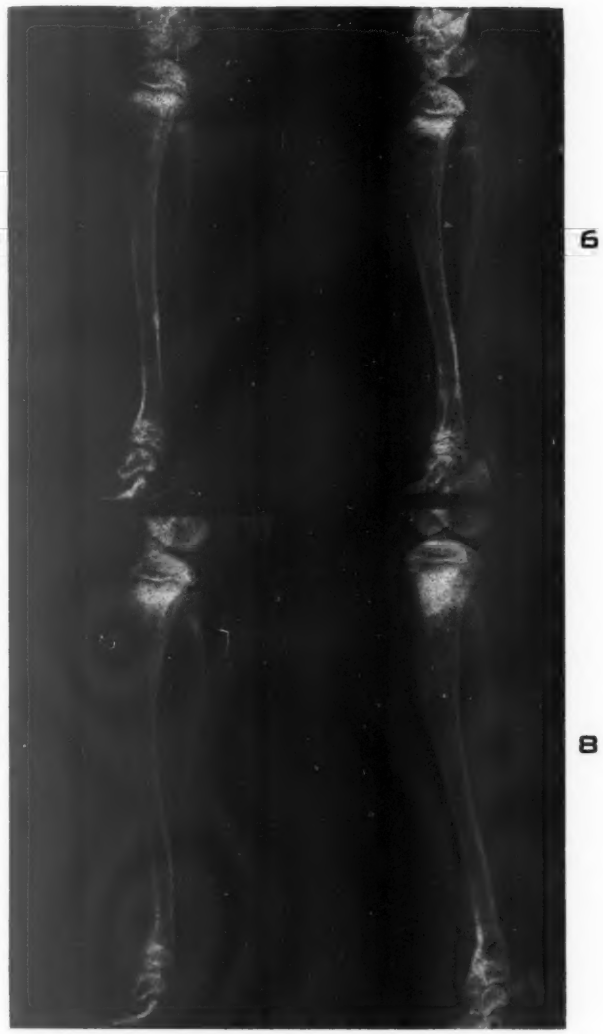
Roentgenograms of the tibiae of rats shown in Figures 1 to 4. The metaphyses increase in length and density with continuous estrogen administration so that the spongiosa is indistinguishable from compact bone. × 2.

FIG. 5. Tibia of the control rat shown in Figure 1. The length of the metaphysis is N.

FIG. 6. Tibia of the estrogen-treated rat shown in Figure 2. The length of the metaphysis is 2 × N.

FIG. 7. Tibia of the estrogen-treated rat shown in Figure 3. The length of the metaphysis is 3 × N.

FIG. 8. Tibia of the estrogen-treated rat shown in Figure 4. The length of the metaphysis is 4 × N.



Budy, Urist, and McLean Effect of Estrogens on Growth of Bone

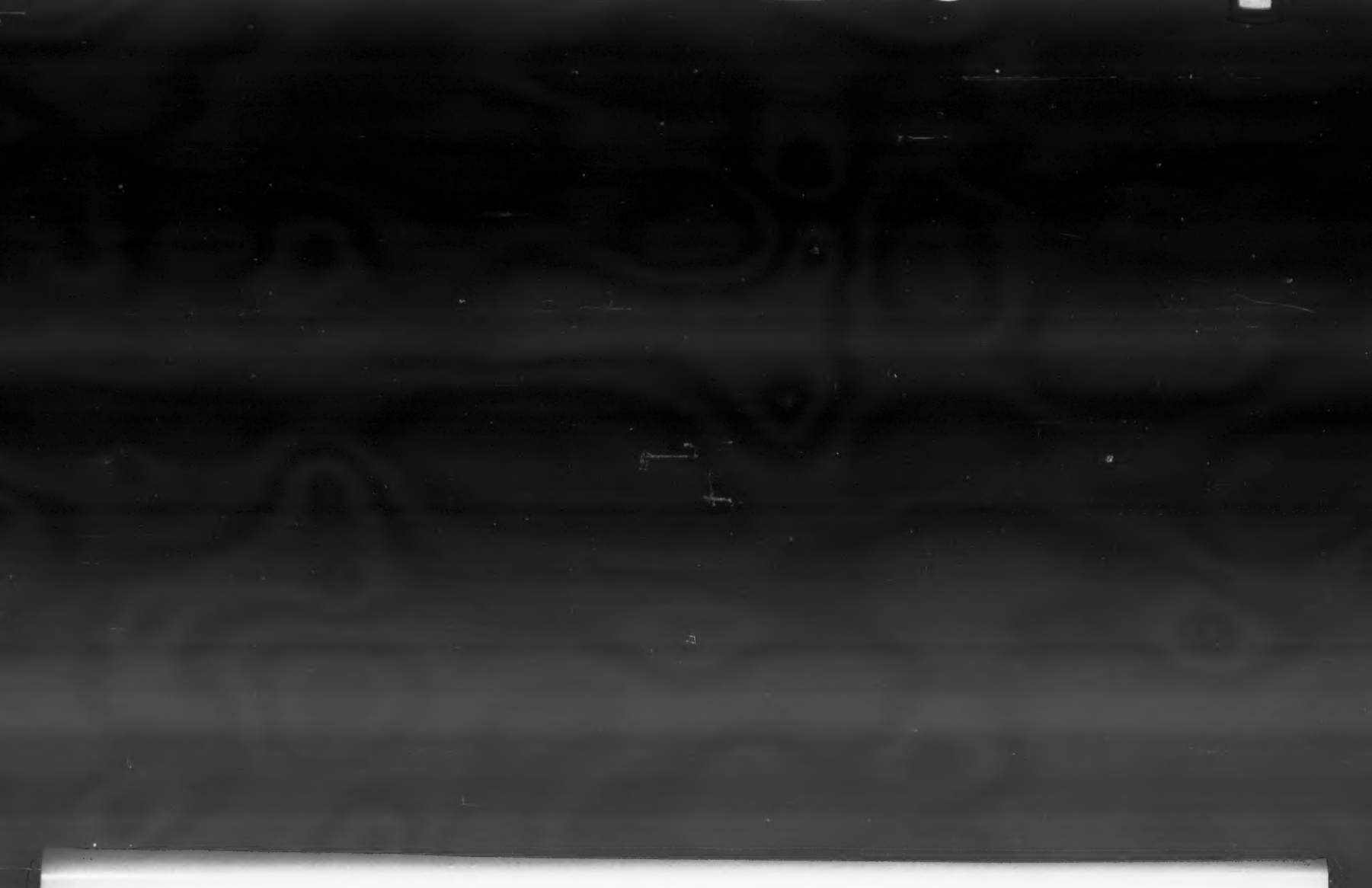
PLATE 188

Roentgenograms of the tibia of a rat treated with a single dose of 4.0 mg. of estradiol benzoate followed by a period of growth. × 4.

FIG. 9. Five weeks after treatment was discontinued, the metaphysis is still influenced by the slowly absorbing estrogen in oil.

FIG. 10. At 8 weeks of recuperation or growth, the metaphysis shows a decrease in density.

FIG. 11. At 14 weeks of recuperation the metaphysis is normal and the effects of the estrogen are no longer apparent.



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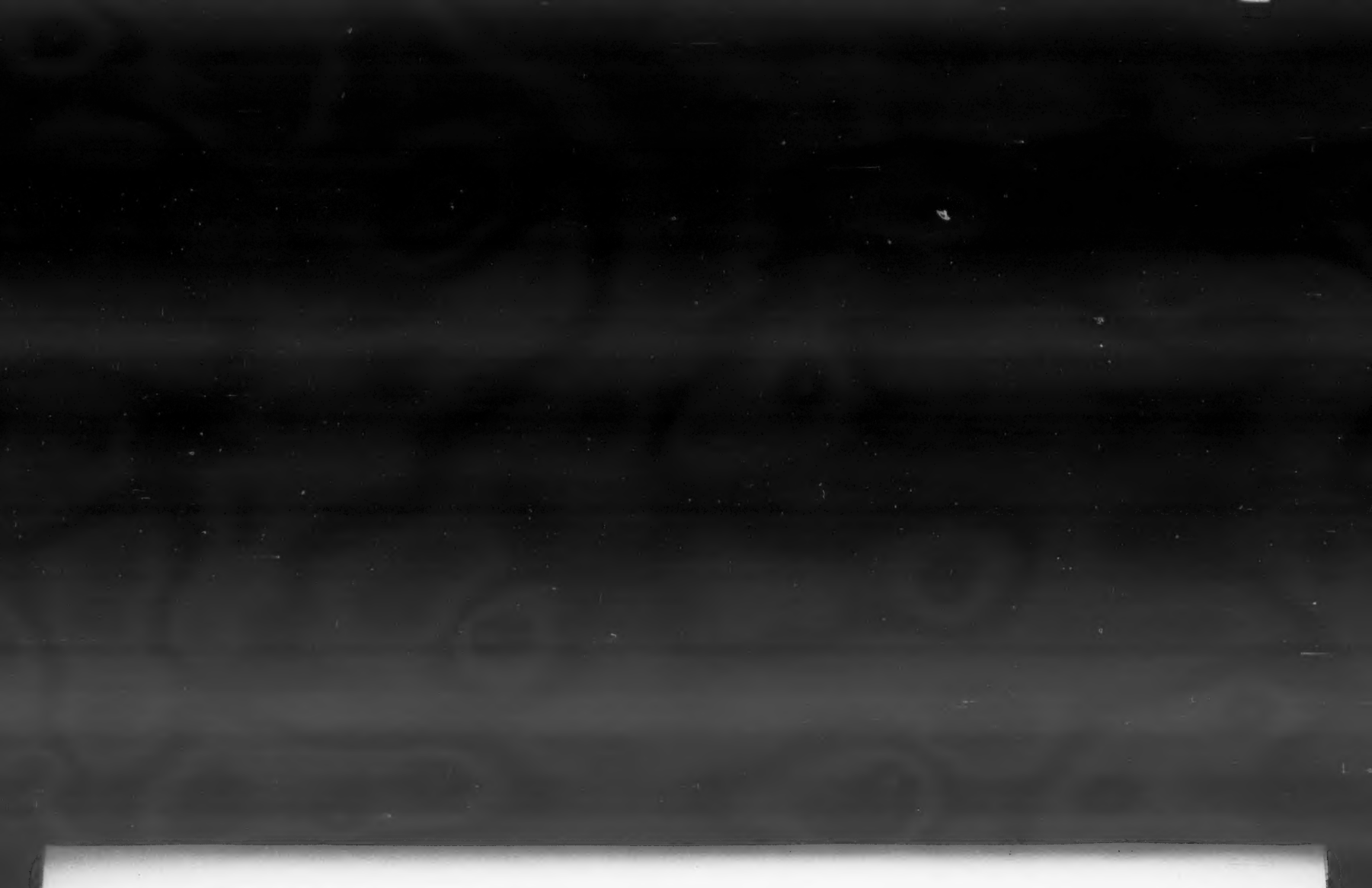
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